Food and Drug Administration

NCTR

National Center for Toxicological Research

Annual Report

FY 2014 – FY 2015

Table of Contents

Preface……i
Strategy……1
Science Advisory Board……2
Interagency Agreements……5
Office of Scientific Coordination……6
NIEHS/National Toxicology Program……9
NCTR/ORA Nanotechnology Core Facility……12
CTP/NCTR Inhalation Core Facility……17
Veterinary Services……19
Cooperative Research and Development Agreements……22
Women's Health Research at NCTR……23
Office of Minority Health……26
Office of the Assoc. Director for Regulatory Activities……28
Division of Biochemical Toxicology……29
Division of Bioinformatics and Biostatistics……36
Division of Genetic and Molecular Toxicology……46
Division of Microbiology……55
Division of Neurotoxicology……68
Division of Systems Biology……74
Projects within NCTR Objective 1.1…… 81
Projects within NCTR Objective 1.2……108
Projects within NCTR Objective 1.3……128
FY 2014 Publications……143
Glossary of Acronyms and Abbreviations……162
Index of Principal Investigators/Division Directors……165

Preface – William Slikker, Jr., Ph.D.

The National Center for Toxicological Research (NCTR) is the U.S. Food and Drug Administration's (FDA) premier laboratory research center. NCTR's primary goal is to support FDA, a critical component of the Department of Health and Human Services (HHS) in its efforts to promote and protect the health of the American public.

NCTR's goals are identified and developed based on goals outlined in FDA's Strategic Priorities document (*Enhance Oversight of FDA-Regulated Products, Improve and Safeguard Access to FDA-Regulated Products to Benefit Health, and Strengthen Organizational Excellence and Accountability*). In support of these Strategic Goals, NCTR scientists work closely with FDA Regulatory Centers in selecting, developing, and evaluating research programs that are needed to address the most pressing regulatory issues.

Once goals are identified, NCTR's scientists develop innovative tools and strategies, and customize safety assessments of chemicals and materials in support of the regulatory process. Comprehensive study designs provide the data required for FDA to make science-based safety decisions. These projects involve coordinating expertise from several disciplines (toxicology, biochemistry, bioinformatics, biostatistics, chemistry, molecular biology, neurotoxicology, microbiology, genomics, nanotoxicology, molecular toxicology, scientific computing, systems biology, and others) to ensure the studies 1) maximize the ability to detect adverse outcomes, 2) provide information on the mechanisms underlying toxicity, and 3) maximize the ability to translate laboratory findings to the improvement and protection of human health. The ongoing work in multidisciplinary, multi-institutional studies evaluating bisphenol A (BPA), nanosilver, and pediatric anesthetics exemplify this approach.

Although studies like the BPA example are designed to identify and resolve specific data gaps required for regulatory decisions, such studies are further designed to be forward-thinking by evaluating the general biology and physiological principles governing the associated biological responses. In studying these more general relationships, NCTR projects provide strategies for evaluating similar toxicants and identifying potential biomarkers of toxicity and safety that will underpin regulatory decisions in the future.

Emerging technologies are deployed in parallel with standardized and traditional approaches to provide real-time comparisons of new tactics with accepted procedures. This also maximizes identification of potential biomarkers of toxicity to be used in further translational research and/or in supporting clinical evaluations of safety and treatment. This synergistic strategy is used in pursuit of the goal to shorten the time to biomarker discovery and qualification, and expedite acceptance of new science for evaluating regulated products in pre- and post-market evaluations.

NCTR strategies include investing in several emerging technologies that both increase the accuracy of safety evaluations and decrease the amount of time required to arrive at sound scientific decisions. A sampling of the newer techniques that are being integrated with established safety-assessment approaches include:

- Minimally invasive imaging approaches using Magnetic Resonance Imaging (MRI), Magnetic Resonance Spectroscopy (MRS), Positron Emission Tomography (PET), and Computed Tomography (CT) for monitoring anatomical and biochemical function in live animals.
- Array and next-generation sequencing-based genomic technologies, along with proteomics and metabolomics capable of measuring hundreds—if not thousands—of simultaneous changes in gene, protein, and metabolite expression; *in silico* approaches.
- Development and standardization of bioinformatic sciences to collect, integrate, and evaluate data.

NCTR enhances FDA's capacity to collect, analyze, and interpret the unprecedented amount of biological data deriving from the omics technologies, high-throughput screening methodologies, the big data revolution, and public-data sharing. NCTR spearheads consortia that reach consensus on standards and approaches for routine use within the regulatory decision process. The MicroArray Quality Control (MAQC) consortium is a primary example of these efforts. MAQC was initiated to establish standards to gather and analyze microarray data, and has now completed its third phase [MAQC III, also known as Sequencing Quality Control(SEQC)] and a series of manuscripts have been published in a special issue of *Nature Biotechnology* to establish procedures and best practice for use of RNA-sequencing technology. In addition, NCTR scientists developed the Liver Toxicity Knowledge Base (LTKB) which has been used in the FDA's review process for drug-induced liver injury.

To meet FDA's growing need for approaches to evaluate nanomaterials, NCTR developed and staffed a specialized core laboratory, in partnership with the Office of Regulatory Affairs, to characterize the nanoscale materials used in safety evaluations and to characterize these materials for use in preclinical safety-assessment studies. This facility has supported many projects at NCTR and other FDA Centers leading to publications on the toxicity and disposition of nanomaterials in cell-based and animal studies. One example is the use of sequential sectioning and imaging using a scanning electron microscope to provide 3-dimensional images of cells at extremely high resolution. A Memorandum of Understanding (MOU) between FDA and the State of Arkansas was established to advance regulatory-science research and build synergy between the five major research universities in the state and NCTR. Supported by the MOU, researchers from the state universities and NCTR investigate new approaches for evaluating the entire lifecycle of nanomaterials.

NCTR's scientists have also established collaborative research projects with the new FDA Center for Tobacco Products (CTP) to address priority research questions that will inform FDA's tobacco product regulatory activities. In support of the research priorities outlined by CTP, NCTR established an inhalation core facility to study the toxicity of tobacco-smoke constituents and a core facility to evaluate the addiction properties of tobacco product constituents.

In addition to newer requirements, NCTR continues to develop rapid technologies for characterizing microbial pathogens, to investigate mechanisms of antimicrobial resistance, and to investigate the virulence of microbes that may enter the food and drug supplies. Microbiome and immune responses, as an aspect of food- and drug-safety research, is being evaluated. This research supports the FDA's emphasis on international food safety that is supported by FDA via its international offices.

The global distribution of products coming under FDA scrutiny requires partnerships in regulatory research and training. FDA is addressing this issue via several programs including NCTR's continued long history of mentoring. NCTR has trained hundreds of scientists from over 45 countries and has expanded its efforts in communications to engage scientists from emerging economies. NCTR fosters national and international research collaborations and communications to promote rapid exchange of theories and emerging science with the promise of improving the quality and effectiveness of regulatory decisions. NCTR also supports the national and international training of scientists in the practices of modern toxicology to propagate the principles of regulatory science which support product-safety evaluation and efficacy.

NCTR formed the annual Global Summit on Regulatory Science (now in its fifth year) as a forum in which to discuss emerging sciences and safety evaluations, to exchange perspectives on regulatory programs, and to provide opportunity for scientific exchange and training. Cooperating and communicating, as well as advancing the principles of regulatory science through the formation of the multinational Global Coalition for Regulatory Science Research, are key factors that continue to enhance domestic and global health.

All of these areas and more are highlighted in this Annual Report. The robust NCTR research portfolio and strategies provide insights into how incorporating regulatory-science research enhances FDA's holistic understanding of toxicological sciences to improve public health.

/s/

William Slikker, Jr., Ph.D.

Director, NCTR

NCTR Vision

The U.S. Food and Drug Administration's National Center for Toxicological Research (NCTR) is a global resource for collaboration — providing consultation, training, and innovative scientific solutions in support of FDA's mission to improve public health.

NCTR Mission

NCTR conducts scientific research to develop and support innovative tools and evaluation of approaches that FDA uses to protect and promote individual and public health.

NCTR Strategic Plan

NCTR's Strategic Plan sets forth our long-term strategic goals and objectives. The plan also details specific actions we are committed to taking as we carry out our mission to provide global leadership and innovative scientific solutions in support of FDA's mission to improve public health. This Strategic Plan charts NCTR's course for the future, focusing on three strategic goals. The three strategic goals NCTR established to accomplish its mission include:

Goal 1: Advance scientific approaches and tools required to support public health.

Goal 1 identifies specific objectives that align with the priorities outlined in FDA's Advancing Regulatory Science Plan. This goal illustrates the importance of maintaining a strong basic-science core; one that provides NCTR the flexibility to address ever-changing research needs.

Goal 2: Promote global interactions in regulatory science research.

Goal 2 defines initiatives that promote NCTR's global activities dedicated to building and strengthening the product safety net around the world.

Goal 3: Improve administrative management and develop new communication materials and methods to support HHS/FDA science goals.

Goal 3 focuses on recruiting and retaining highly qualified scientists and staff, improving business processes, and extending the reach of NCTR's internal and external communications.

The NCTR Strategic Plan can be found on the FDA website at: www.fda.gov/NCTRStrategicPlan.

Science Advisory Board

Function

The Science Advisory Board (SAB) advises the NCTR Director in establishing, implementing, and evaluating the scientific-research programs conducted at NCTR. NCTR conducts innovative scientific research that assists FDA in fulfilling its regulatory responsibilities. Through site-visit reviews and annual meetings, NCTR's SAB provides an extra-agency scientific review of the research programs at the Center. The recommendations of the SAB are critical to the scientific rigor of the studies conducted. Members of the SAB and the SAB Chair are selected by the FDA Commissioner, or designee, from among leading authorities in fields related to the research done at NCTR.

FY 2014 Accomplishments

The NCTR SAB held a meeting on January 29-30, 2014 to provide feedback on the scientific achievements and future plans of NCTR. The Director of NCTR welcomed the SAB members, FDA Center representatives, and NCTR participants and presented a "State of the Center" address. Each of the six research divisions at NCTR presented some of the work being done and elicited feedback from the SAB. The Co-Chairs of the Division of Microbiology Subcommittee presented the Site Visit Report which was adopted by the full SAB. Additional speakers on day one included a representative from the National Toxicology Program. The focus on day two was on bioinformatics. Speakers included representatives from NCATS/NIH, CBER, CDER, CVM, CTP, ORA and a representative from the Arkansas Bioinformatics Consortium initiated in 2012 under the auspices of the FDA/State of Arkansas Memorandum of Understanding.

SAB Membership Roster

CHAIR:

Martin A. Philbert, Ph.D.
Term: 06/05/13 – 06/30/16
Expertise: Neurotoxicology
Dean and Professor of Toxicology
School of Public Health
The University of Michigan
1415 Washington Heights
Ann Arbor, MI 48109-2029

DESIGNATED FEDERAL OFFICER:

Donna L. Mendrick, Ph.D.
National Center for Toxicological Research
10903 New Hampshire Avenue
Building 32, Room 2208
Silver Spring, MD 20993-0002
Tel: 301-796-8892

MEMBERS:

Susan P. Felter, Ph.D.
Term: 10/7/14 – 06/30/18
Expertise: Cancer Risk Assessment
Principal Toxicologist
Global Product Stewardship
Proctor & Gamble
8700 Mason Montgomery Road
Mason, OH 45040

Jay Gandy, Ph.D.
Term: 07/01/12 – 6/30/16
Expertise: Risk Assessment/Regulatory Science
Professor & Chair
Department of Environmental & Occupational Health
Fay W. Boozman College of Public Health
University of Arkansas for Medical Sciences
4301 W. Markham St., #820-11
Little Rock, AR 72205

Diwaker Jain, MD FACC FRCP FASNC
Term: 10/1/14 – 6/30/18
Expertise:
Imaging/Cardiotoxicity
Pharmacology
Professor of Medicine (Cardiology)
Director of Nuclear Cardiology
Westchester Medical Center
Valhalla, NY 10595

Pamela J. Lein, Ph.D.
Term: 10/10/14 – 6/30/18
Expertise: Developmental Neurotoxicology
Vice Chair, Department of Molecular Biosciences
Professor of Neurotoxicology
UC Davis School of Veterinary Medicine
1089 Veterinary Medicine Dr.
2009 VM3B
Davis, CA 95616

Suresh D. Pillai, Ph.D.
Term: 01/28/14 – 6/30/17
Expertise: Microbiology & Immunology
Professor of Microbiology
Director, National Center for Electron Beam Research Room 418B, Kleberg Center
Texas A&M University
College Station, TX 77843

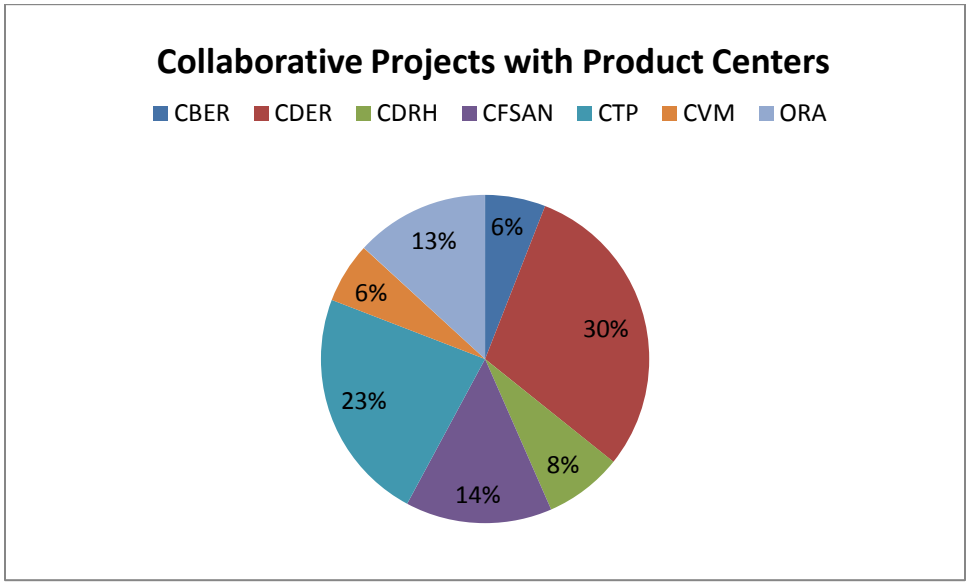
David Warheit, Ph.D.
Term: 07/01/12 – 06/30/16
Expertise: Inhalation Toxicology/Nanotoxicology
Senior Research Toxicologist
Acute and Developmental Toxicology Division
E.I. du Pont de Nemours & Co., Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
1090 Elkton Road
P.O. Box 50
Newark, DE 19714

Katrina M. Waters, Ph.D.
Term: 06/25/13 – 6/30/17
Expertise:
Bioinformatics/Systems Toxicology
Staff Scientist
Computational Biology & Bioinformatics
Pacific Northwest National Laboratory
902 Battelle Boulevard
P.O. Box 999, MSIN J4-33
Richland, WA 99352

CONSUMER REPRESENTATIVE:
Heidi Moline, M.P.H.
Term: 8/05/11 – 06/30/15
Scientific Integrity Program
Union of Concerned Scientists
1825 K Street NW, Suite 800
Washington, DC 20006

NCTR Advances Research Through Outreach and Collaboration

NCTR has actively sought and participated in collaborative, cooperative partnerships with other scientific and regulatory organizations, especially where the partnership augments the mission of NCTR and FDA through the use of NCTR's unique resources. These opportunities to leverage resources, both public and private, enable NCTR to address questions of common concern to both FDA and the collaborating entity. These partnerships have led to substantial research advances that have resulted in significant improvements in long-term public health, such as regulatory guidance, mechanistic understanding, and advanced methodology. NCTR is working with nine academic institutions and also works actively with every one of the FDA Product Centers on various research efforts. Of our 231 active projects, NCTR collaborates with at least one Product Center on 118 of those. Shown below is the percentage of projects per Center of the 118 collaborative projects in place as of March 2015.



Interagency Agreements

An Interagency Agreement (IAG) is a formal financial partnership between multiple government agencies. NCTR has been fortunate in establishing IAGs with several government agencies to conduct research on problems of common interest to FDA and the collaborating agency. The most significant, in terms of size, is the IAG between FDA/NCTR and the National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP).

Office of Scientific Coordination (OSC)

Paul C. Howard, Ph.D., Director
870-543-7672
Paul.Howard@fda.hhs.gov

Introduction

The Office of Scientific Coordination (OSC) has as its purpose *"… to enable the research mission of NCTR and FDA by providing the support necessary for conducting toxicology studies."* This includes support of animal-based toxicology studies, pathology, nanotechnology characterization and detection, inhalation toxicology, and management of the Interagency Agreement (IAG) with the National Toxicology Program (NTP) of the National Institute of Environmental Health Sciences (NIEHS). In order to accomplish this goal, many of the key support staff and operations are located in OSC.

The OSC oversees the IAG between NTP and NCTR, and has the responsibility of managing the IAG, communicating study progress to NTP and FDA, coordinating the generation of final reports, and generating background documents for many of the FDA-nominated chemical substances to NTP. The Director of the OSC serves as the liaison between FDA and NTP.

The following components of OSC are described elsewhere in this report:

- National Institute of Environmental Health Sciences/National Toxicology Program
- Veterinary Services Staff
- NCTR/ORA Nanotechnology Core Facility
- CTP/NCTR Inhalation Toxicology Core Facility

Pathology Services Contract

NCTR maintains an on-site pathology contract for veterinary pathology and histopathology services, and the Contract Officer Representative resides in OSC. This service is a critical component of conducting toxicological studies. The contractor maintains a staff of five board-certified veterinary pathologists and a highly trained staff that provide NCTR with services including: necropsy, clinical pathology, histopathology slide preparation, rigorous pathology examination, and complete histopathology and pathology reports for each study. The conduct of the pathology services follows the guidelines suggested by veterinary pathology organizations, including the *Specifications for the Conduct of Studies to Evaluate the Toxic and Carcinogenic Potential of Chemical, Biological, and Physical Agents in Laboratory Animals for the National Toxicology Program*.

The pathology services additionally include translational and applied research that must be performed with rigid adherence to standard operating procedures, including the conduct of operations that meet the requirements of Good Laboratory Practice (21CFR58).

The Pathology Services Contractor provides specialized analytical services which include:

- Immunohistochemistry proliferation assays
- BrdU immunohistochemistry
- Ki-67 (MIB-5) immunohistochemistry
- Proliferating cell nuclear antigen (PCNA) immunohistochemistry
- *In situ* hybridization for histone mRNA apoptosis assays
- *In situ* hybridization PCR-Solution PCR
- *In situ* RT-PCR
- Laser-capture microdissection
- Image analysis using PC-based Optimus and Image Pro Plus image analysis software
- Imaging system: Virtual Microscopy/Pathology System (ScanScope) for digital storage of microscope slides at diagnostic resolution for local and remote diagnostic collaboration

The Pathology Services Contractor in FY14 supported 46 NCTR research protocols through the necropsy of 3,887 animals. This resulted in the trimming, processing, and embedding of 34,560 histopathology samples, generating 25,452 slides for histological examination. In support of the research studies 5,478 immunohistochemistry slides were prepared. Additionally, clinical chemistry (20,644 samples) and special pathology-related analyses (4,311 assays) were conducted as requested in research protocols.

Equipment Maintenance and Repair Contract

NCTR maintains a contract for equipment maintenance and repair, and the Contract Officer Representative resides in OSC. This contract supports research in three specific areas. The first is the routine preventative maintenance and calibration of equipment essential for the conduct of research. In FY14 this included preventative maintenance on 919 instruments on the campus, including preventative maintenance and calibration of 381 balances to ensure they provide accurate measurements for research. The second is the repair of equipment supporting research that is not on a service agreement with the manufacturer. Minor equipment, such as balances, centrifuges, vacuum system, spectrometers, and chromatography systems, are repaired by the contractor to maximize utilization of aging equipment. The third is the manufacture of minor equipment to support customized research needs. This may involve the manufacture of a component of an old system when parts are no longer available, or the synthesis of new devices customized for specific applications, such as behavioral testing devices.

Experimental Support

Two groups in OSC support animal-based research at NCTR. The Experimental Support Liaison Staff review the research protocols, develop an animal-use plan, review this plan with the study scientist, and interact with the computer system that collects animal data (Multigen), where they input the study design into the system. This critical component of protocol execution communicates through the computer system and directs the animal-care contractor daily operations that involve animals, animal care, and animal dosing.

The Document Support Group specialists accomplish three specific tasks in support of NCTR research: Validation and Documentation regarding animal data systems (*e.g.* Multigen); documentation of Standard Operating Procedures (SOPs) for all operations of the animal data systems and computer-based systems; development of qualification and validation documents for hardware and software involved in the animal data system.

FY 2015 Plans

In FY 2015 the Office of Scientific Coordination will continue to support the research at NCTR in each of the respective areas including support of the NTP Interagency Agreement, Veterinary Services, Nanotechnology Core Facility, experimental support, Pathology Contract support, and equipment maintenance and repair.

National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP)

Paul C. Howard, Ph.D., Associate Director, Office of Scientific Coordination
870-543-7672
Paul.Howard@fda.hhs.gov

The National Toxicology Program (NTP) is an interagency program established in 1978 to coordinate toxicology research and testing across the Department of Health Human Services. The program was created to strengthen the science base in toxicology, to develop and validate improved testing methods, and to provide information about potentially toxic chemicals to health regulatory and research agencies, scientific and medical communities, and the public. NTP consists of three core agencies that provide support for NTP activities: National Institute of Environmental Health Sciences (headquarter facility; NIEHS/NTP), U.S. Food and Drug Administration's National Center for Toxicological Research (FDA/NCTR), and Centers for Disease Control and Prevention's National Institute for Occupational Safety and Health (CDC/NIOSH).
In support of the NTP mission, NIEHS/NTP and FDA/NCTR established an Interagency Agreement (IAG) in 1992, facilitating the conduct of toxicology studies on chemicals or substances nominated to NTP, that may be under the regulatory purview of FDA, to be studied using the unique resources and facilities at NCTR. The IAG-supported toxicology program provides FDA regulatory scientists with the opportunity for input into the toxicology-study design and dose selection to maximize the utility of the data for risk assessment. The program also provides FDA with the toxicology data that is needed for safety assessment of some FDA-regulated products.

The success of this IAG has led to 22 years of collaborative toxicity testing on compounds of interest to FDA and NTP. The IAG program has led to the investigation of toxicity assessment and mechanism-of-action studies of compounds in many classes of chemicals including:

- food contaminants
- cosmetics
- endocrine-disruptor compounds
- food cooking by-products
- dietary supplements
- drugs
- anesthetics

There is a significant probability that humans exposed to the above listed classes of compounds will also be exposed to sunlight. In order to test the effect of sunlight on the toxicological risk of chemicals, the IAG supported the development of a facility (NIEHS and FDA Phototoxicology Research and Testing Laboratory) capable of conducting

animal toxicology studies in the presence of simulated solar light or portions of the sunlight spectrum such as UVB and UVA. These studies either test the acute effects of sunlight on chemical toxicity (phototoxicity) or the effects following long-term exposure of sunlight and chemical (photocarcinogenesis and photococarcinogenesis) studies.

Nanotechnology is the manipulation of matter at the near atomic scale, and materials developed with this technology have unique properties not found in bulk or larger particles. These materials require unique methods for proper characterization for toxicology studies. NCTR together with NIEHS/NTP and FDA's Office of Regulatory Affairs (ORA) have developed a core facility in nanotechnology (NCTR/ORA Nanotechnology Core Facility), where nanoscale materials are characterized for toxicology studies. The equipment and procedures are in place to detect nanoscale materials *in vitro* and *in vivo* in support of toxicological studies.

All toxicology studies conducted under the IAG are designed with input from many sources including FDA regulatory scientists, scientists from NCTR, NIEHS, and other federal agencies, and invited subject-matter experts. The IAG utilizes resources from public funds and exceptional scientific expertise to provide the best possible assessment of product safety through toxicological and mechanistic studies.

The IAG fulfills one of the strategic goals of NCTR (Strategic Goal 1, *Advance Scientific Approaches and Tools Required to Support Public Health*) through the conduct of toxicology studies that will provide FDA with appropriate data for quantitative risk assessment of compounds. In addition, the studies are accompanied with mechanism-of-action and biomarker studies. These allow scientific understanding of the toxicology process and provide information for translation of the safety assessment to humans.

The NCTR Office of Scientific Coordination (OSC) is responsible for managing the IAG, communicating study progress to NTP and FDA, coordinating the generation of the final reports, and generating background documents for many of the FDA-nominated chemical substances to NTP. These nomination documents are complete literature reviews of the use, pharmacokinetics, human exposure, and toxicity of the nominated substance. OSC will continue to produce these review documents to support FDA's need for assistance from NTP in understanding the risk of chemical substances to human health.

Toxicological studies on numerous compounds have been supported since 1992. Many of the compounds are listed below with the nominating or contributing FDA Center in parenthesis.

- Acrylamide (CFSAN)
- α- and β-hydroxy acids (CFSAN)
- AIDS therapeutics (Zidovudine, Nelfinavir, Nevirapine, Lamivudine)
- *Aloe vera* (CFSAN)
- Bisphenol A (CFSAN)
- Bitter orange, *Citrus aurantium* (CFSAN)
- Cellular telephone radiation (CDRH)
- Chloral hydrate (CFSAN)
- Di-(2-ethylhexyl)phthalate (CBER, CDRH)
- Ethinyl estradiol (CDER)
- Fumonisin B1 (CFSAN)
- Furan (CFSAN)
- Genistein (CFSAN)
- Glucosamine/Chondroitin (CFSAN)
- Goldenseal, berberine (CFSAN)
- Ketamine (CDER)
- Malachite green (CVM)
- Melamine with cyanuric acid (CVM)
- Nanoscale silver (FDA)
- Nonylphenol (CDER)
- Oxybenzone (CDER)
- Permanent makeup pigments (CFSAN)
- Retinyl palmitate (CFSAN)
- Riddelliine (CFSAN)
- Triclosan (CDER)
- Urethane/Ethanol (CFSAN)
- Usnic acid, *Usnea* lichen (CFSAN)

NCTR/ORA Nanotechnology Core Facility (NanoCore)

Anil K. Patri, Ph.D., Director (since Aug 2014)
Paul C. Howard, Ph.D., Director (until Aug 2014)
Anil Patri: 870-543-7508
Anil.Patri@fda.hhs.gov

Introduction

The NCTR/ORA Nanotechnology Core Facility (NanoCore) at Jefferson Laboratories is a joint effort by NCTR and the Office of Regulatory Affairs' (ORA) Arkansas Regional Laboratory (ARL) with a mission to:

"Provide the nanotechnology technical expertise and capability to support nanotechnology-based regulatory research and surveillance needs of NCTR, ORA, FDA, and government agency partners."

Nanotechnology is a multidisciplinary field, drawing from applied and device physics, material science, supramolecular and polymer chemistry, interface and colloidal science, and engineering (chemical, mechanical, biological, and electrical). This field involves the manipulation of matter at the atomic level to create new materials called nanomaterials. Nanomaterials are typically defined as those with one size domain (length-width-height) between 1 and 100 nm (0.001 to 0.1 micrometer), where the size results in the presence of properties that are not found in the same material in larger sizes; however, the upper bounds (i.e. 100 nm) is not well-established in regulations. The detection and characterization of nanomaterials requires specialized equipment and procedures that are not routine in either toxicology or analytical chemistry laboratories.

The NanoCore was developed to fill a need that arose within the toxicology research community, that is, a need to properly determine the physical and chemical characteristics of nanomaterials to be tested, understand the behavior of these particles in the dosing solutions for *in vivo* studies and media for *in vitro* studies, and the ability to detect these materials or their break-down products in cells and tissues. As a result, the NanoCore has acquired the equipment and technical expertise to adapt and develop procedures to address the following:

1. Characterization of the nanomaterials used in toxicology and other studies to include:

    - Size and size distribution
    - Shape
    - Agglomeration and aggregation (i.e. particles collecting into bigger particles)

- Concentration in solutions (mass, particle number, and surface area)
- Overall composition (i.e. elements and crystal state)
- Purity, elemental and organic (e.g. endotoxin contamination)
- Surface area
- Surface charge (zeta potential)
- Stability (isolated nanoparticle and when in solution)
- Dosimetrics (i.e. measurements of the dose delivered to the cell)
- Detection of nanomaterials in biological and physical matrices.

2. Detection of the nanomaterials in *in vitro* or *in vivo* toxicology assays. This requires knowledge of unique features of the test nanomaterial and developing/adapting existing methodologies to provide sensitive assays for detection. One critical starting point to any study on nanomaterials is characterization of the test article and determination of its behavior in the test environment (e.g., suspension in solution, in food matrix, in water). There is strong agreement that test articles should be characterized for many properties, including average particle size, agglomeration, shape, chemical composition, purity, crystallinity, stability, sterility, endotoxin presence, surface area, chemistry, and surface charge. The NanoCore supports investigators by providing the appropriate equipment, standard operating procedures, standards, and personnel to either conduct these characterizations or train laboratory personnel on how to conduct the analyses.

FY 2014 Accomplishments

In FY 2014, the NCTR staff of the NanoCore expanded its role in toxicology studies conducted at NCTR and other FDA agencies, supporting the characterization of nanomaterials (e.g., nanoscale silver, gold, iron oxide, titanium dioxide, zinc oxide) used in biodistribution and toxicology studies. The electron microscopy component of the NanoCore expanded its capabilities by obtaining a Zeiss Merlin scanning electron microscopy (SEM) for high-resolution imaging of the nanomaterials, and is equipped with X-ray Energy Dispersion Spectral (EDS) detectors for the analysis of the elemental composition of the sample being imaged. This SEM complements the existing Zeiss Merlin SEM that is equipped with a serial block-face sectioning device (Gatan) that allows sequential sectioning and imaging of a sample. This allows the instrument to section and image in the Z-plane through a sample, creating a stack of images resulting in a 3-dimensional image of the sample. This capability allows for the imaging of the ultrastructure of samples and the 3-dimensional location of nanomaterials in biological tissues. This latter device led to the imaging of many samples in FY 2014 in support of research projects, most notably the imaging of rat brain hippocampus from control and animals treated with ketamine. This led to the observation of mitochondrial fission/fusion channels and disappearance of this communication as the ketamine treated cells entered into apoptosis. This work corroborated the analysis of the

mitochondria structure in the tissues using Transmission Electron Microscopy where it was quantified that ketamine treatment resulted in progression into apoptosis, which confirms histochemical observation of caspase expression. This study demonstrated the power of 3-dimensional stacked imaging of tissues at electron microscopic resolution, in resolving ultrastructural biology questions.

These Zeiss SEMs are complemented by a Jeol SEM and two Jeol transmission electron microscopes (120 kV and 200 kV). This led to the processing and imaging of over 1,000 samples in 2014 in support of research projects at NCTR and other FDA centers.

The NanoCore additionally has a Particle Evaluation and Analytical Spectroscopy (Nano-PEAS) team with the responsibility to conduct the analyses of particle size, concentration, purity, and detection in biological matrices using a variety of techniques. The Nano-PEAS team continued to develop techniques to support multiple research projects from NCTR and FDA investigators and allow researchers to:

- Quantify the particle size of nanomaterials in solution (e.g., dynamic light scattering, particle-tracking analysis, atomic force microscopy)
- Quantitatively analyze nanoparticles in biological samples [e.g., inductively coupled plasma mass spectroscopy (ICP-MS), darkfield microscopy with hyperspectral imaging, confocal Raman spectroscopy]
- Detect unique carbon-containing nanomaterials in biological samples (e.g., Raman spectroscopy).

These advances in technology not only enabled the NanoCore team to support ongoing research, they also held a “hands-on” training session for FDA scientists on the strengths and weaknesses of various equipment and techniques on the determination of the size of nanomaterials. This course has been highly successful, receiving outstanding reviews from the participants which represent each FDA Center.

A notable project that was completed in FY14 included the characterization of nanoparticles in spray sunscreens. This request from FDA’s Center for Drug Evaluation and Research (CDER), led to the development of a methodology to extract the nanoscale (and larger) particles from the sunscreen liquid, perform elemental analysis to determine the elemental composition (inductively coupled plasma-mass spectroscopy, ICP-MS), determine particle size in liquid suspensions, and confirm primary particle sizes using transmission and scanning electron microscopy. The results of this survey of 15 commercial products led CDER to the selection of one product for further testing at the National Institute for Occupational Safety and Health for lung deposition following particle inhalation.

The NanoCore also participated in a research consortium with research universities in Arkansas by providing size, purity, and elemental composition analysis of graphene to be used in toxicology studies at the universities and NCTR. Elemental analysis led to the

observation that “pristine” graphene purchased for the studies was highly contaminated with many elements (i.e. Ca, K, Mn, etc.).

As examples, NanoCore personnel also participated in support of research studies in several NCTR divisions, providing critical characterization, stability, and detection analyses. These included studies on:

- the vaginal penetration of nanoscale gold in rat vaginal tissue (*Nanoscale Materials in Feminine Hygiene Products Targeted to Women: A Rat Model of Mucosal Penetration*)
- dosing of rats with nanoscale gold, silver and silica and *in vivo* challenge with bacterial infection (*Does the durable nanoparticle bioaccumulation in macrophages increase susceptibility to bacterial infection*)
- support of *in vitro* studies on the genotoxicity of nanoscale silver in standard genotoxicity assays (*Do engineered silver nanomaterials (Ag-ENMs) varying by size and coatings behave differently than bulk silver in their ability to induce genetic damage*).

One small project is having large implications on determining the particle status of nanomaterials in biological fluids. In this project, investigators were able to detect the agglomeration status of nanoscale gold in whole blood by examining the plasmonic resonance using UV-VIS and Raman spectroscopy. Expansion of this project could lead to understanding of nanoparticle behavior *in vivo*.

These accomplishments supported the NCTR Strategic Plan, specifically Goal 1: *Advance Scientific Approaches and Tools Required to Support Public Health* and Objective 1.1: *Integrated Product Assessment*.

FY 2015 Plans

The plans for the NanoCore in FY 2015 include addressing specific research needs that align to NCTR Strategic Plan Goal 1 (*Advance Scientific Approaches and Tools Required to Support Public Health*). The NanoCore will continue working with investigators to characterize the nanomaterials used in their toxicology studies. This includes continued development of methods to better characterize the behavior of nanoparticles in solutions and evaluation of the chemistry at the surface of the nanomaterials. The NanoCore will continue to detect and quantify the nanomaterials in the biological samples derived from toxicological studies, and will continue to develop new methods, or adapt existing methods, to detect single particles of nanomaterials in biological matrices. This includes electron microscopic methods, single particle ICP-MS, and other spectroscopic techniques, such as Raman spectroscopy. These goals and objectives are consistent with the FDA Strategic Objective 2.1 *(Increase Regulatory Science Capacity To*

Effectively Evaluate Products), generating data to support FDA regulatory decisions on the safety of nanomaterials.

Research projects that started in late FY14 and that will be conducted in FY15 include studies on the complement activation various species by soft- (*e.g.* liposomes, dendrimers) and hard- (*e.g.* TiO2) nanoscale materials (*Complement Assays for the Detection of Immuno-Sensitizing Activity of Nanomaterials*). A complement activation assay was developed at Washington University, and NCTR is serving as the beta-test site for this assay. Since many nanomaterials are administered by intravenous injection, understanding whether these nanomaterials will activate complement and be sequestered into large aggregates, will play a key role in understanding the nanoparticle disposition and ability to reach the target tissue.

A second research project that started in late FY14 and will continue through FY15 is a study on the pharmacokinetics of liposomal-based doxorubicin, and the effect of size and internal liposomal salt content on pharmacokinetics (*Physiologically-Based Pharmacokinetic (PBPK) Modeling of Nanomedicine; Building Clinically Relevant Standards for FDA-Regulated Nanoparticulate Drug Products*). This project is in collaboration with the Center for Drug Evaluation and Research.

The NanoCore will continue to support studies at FDA's NCTR, Office of Regulatory Affairs, Center for Device and Radiological Health, Center for Drug Evaluation and Research, Center for Food Safety and Nutrition, Center for Veterinary Medicine, local universities (through FDA and a Memorandum of Understanding with the State of Arkansas), and others.

Center for Tobacco Products (CTP)/NCTR Inhalation Toxicology Core Facility (InhaleCore)

Shu-Chieh Hu, Ph.D., Director
870-543-7513
Shu-Chieh.Hu@fda.hhs.gov

Introduction

The CTP/NCTR Inhalation Toxicology Core Facility (InhaleCore) at Jefferson Laboratories is a joint effort by FDA's NCTR and Center for Tobacco Products (CTP) to provide technical expertise in applied research via the inhalation route. This joint effort provides research within FDA in the field of inhalation toxicology for the purpose of supporting the authorities within the Family Smoking Prevention and Tobacco Control Act to protect public health.

The lung is the primary portal of entry for cigarette smoke, which is a combustion product containing thousands of chemical constituents, many of which are toxicants, carcinogens, and addictive compounds. In order to determine the adverse health risks associated with humans using tobacco products, *in vivo* ('within the living') inhalation toxicology studies are therefore warranted. Inhalation toxicology studies are necessary to evaluate the dose-response toxicity of chemicals that are found in tobacco, or that form during the combustion process. The reason for establishing the InhaleCore Facility in FY 2013 was to conduct these studies using the advantage of NCTR's unique capabilities in toxicological research. In collaboration with CTP and NCTR scientists, the InhaleCore researchers study animal biological responses using various endpoints after they are exposed in a well-defined environment via nose-only inhalation. These testing procedures are always in compliance with the Good Laboratory Practice (GLP; 21CFR58), as well as international test guidelines (e.g., Organization for Economic Co-operation and Development, OECD). The research outcomes provide data to inform the understanding and quantification of the adverse health risks associated with humans using tobacco products, supporting the FDA mission of regulating tobacco products.

FY 2014 Accomplishments

The InhaleCore facility is equipped with four TSE Systems, Inc., flow-past, nose-only inhalation exposure systems, including associated instruments for the generation of vapor, particulate, or aerosol test articles; optical and physical instruments for the quantification of test article concentration or particle size; medical grade compressed air source; and vacuum pumps. The system operates as an "enclosed system within an enclosed system" for the safety of personnel. The facility was installed and validated in FY 2013.

During FY 2014, InhaleCore researchers initiated a pharmacokinetic study of nicotine-derived nitrosamine ketone (NNK), a tobacco-specific nitrosamine which occurs naturally in cigarette smoke and is one of the carcinogens that contributes to the induction of lung cancer in humans exposed to tobacco smoke. In separate studies, animals were dosed with NNK either by oral gavage, intraperitoneal injection, or inhalation, and NNK and metabolites quantified in biological fluids and tissues. The results provide biodistribution data of NNK following inhalation exposure, which will inform the design of subsequent toxicological studies of inhaled NNK.

FY 2015 Plans

In FY 2015, the CTP/NCTR InhaleCore will focus on completion of the inhalation toxicology studies of NNK. This will include conduct of a repeated-dose inhalation toxicity study and 90-day subchronic inhalation toxicity study.

These studies will be followed by a repeat of pharmacokinetic and inhalation toxicity studies of interest to CTP.

The FY 2014 accomplishments and FY 2015 plans support the NCTR Strategic Plan, specifically Goal 1: *Advance Scientific Approaches and Tools Required to Support Public Health* and Objective 1.1: *Integrated Product Assessment.*

Veterinary Services

Jefferson Carraway, D.V.M., Director, Veterinary Services Staff (retired)

The Veterinary Services Staff (VSS) provides professional and technical support for all animal-related research projects at NCTR. VSS administers NCTR's Animal Care and Use Program, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) since 1977. Included within VSS is the on-site Diagnostic/Microsurveillance Unit.

Three veterinarians, all certified by the American College of Laboratory Animal Medicine (ACLAM), one also certified by the American Board of Toxicology, and all of whom hold research degrees in addition to Veterinary Medical degrees, are charged with ensuring that healthy animals are available for research projects, providing veterinary care as needed, training research staff, and participating in projects requiring veterinary expertise.

A member of the VSS staff is the Contract Officer Representative with oversight of the contracted services for animal husbandry, diet preparation, and veterinary care of nonhuman primates. The animal care contract workforce is stable, highly trained and skilled, and boasts a high percentage of certified employees in their respective disciplines. The VSS Supervisory Veterinarian is a member's of NCTR's Institutional Animal Care and Use Committee (IACUC), serving as the Institution's Attending Veterinarian.

VSS oversees the operation of five animal facilities on campus consisting of over 114,000 square feet of space dedicated to providing state-of-the-art housing and care of research animals. A variety of housing options are available for rodent models including ventilated rack systems and automatic watering systems. A highly trained and American Association for Laboratory Animal Science (AALAS)-certified animal care staff provides a wide variety of husbandry and technical services in support of NCTR's AAALAC-accredited Animal Care and Use Program. A staff of four microbiologists provides superior on-site microsurveillance and diagnostic services, ensuring the animals used in research projects at NCTR are free of pathogens that could compromise the research program. Monitoring of animal facility sanitation/sterilization practices, feed, water, and bedding prevents research animal exposure to microbial pathogens via these sources. In addition, this group supports the provision of exceptional veterinary care via on-the-spot diagnostic services (bacteriology, serology, parasitology, and molecular biology) facilitating the prompt development of strategic treatment actions.

The Diet Preparation Facility is a well-equipped, large-scale formulation services unit. All animal diets received at NCTR are processed through the Diet Preparation Facility. The

majority of dosed diets, dosed water, gavage solutions, and topical creams used in experiments performed at the Center are prepared in this facility. Dosed-feed production capability is 200,000 kg per year. Diets can be mixed with test articles in solution or solid state in concentrations as low as 0.1 parts-per-billion. In addition, test articles can be mixed in the animals' drinking water to exacting standards in concentrations as low as one microgram per milliliter.

VSS provided oversight and management of all NCTR laboratory animal facilities. Staff personnel were responsible for breeding, rearing, acquiring, and quarantining all experimental animals used on-site. Personnel submitted annual reports (USDA, OLAW, AAALAC) assuring compliance with federal regulations relative to our Animal Care and Use Program and participated in semi-annual program reviews, facility inspections, and experimental protocol reviews as part of the NCTR IACUC proceedings.

The Veterinary Care program, administered through VSS, provided veterinary medical care and surgical services to NCTR's research animals, including oversight of policies and procedures for animal procurement and transportation, preventive medicine, health and genetic monitoring, environmental enrichment, surgical protocols, anesthesia of laboratory animals, pain management, and euthanasia. Veterinarians also served as Principal Investigators or Co-Investigators on several protocols including rodent breeding operations, animal procedures training, and the sentinel animal program.

A significant accomplishment this year was the initiation of cryopreservation projects for one transgenic mouse strain and two rodent strains from our breeding colony that were established over 35 years ago. These projects provide preservation against the loss of several invaluable animal models.

Animal Care/Diet Preparation/Veterinary Care Services Contract

During FY 2014, contract personnel supported an average daily census of 32 experiments. These experiments entailed husbandry services for an average daily census of 4000 rodents, 169 rhesus monkeys, and 490 zebrafish. A variety of technical procedures were performed on many experiments including tattooing, tumor palpations, biological sample collections, administration of test articles, oral gavage (224,559 procedures), behavior assessments on rats and rhesus monkeys (37,561 measurements), application of topical-dosed creams (398,715 applications), rodent breeding operations, quarantine of rodents, physical and pregnancy examinations of rhesus monkeys, microchip implantations, and humane euthanasia. An on-site rat-production operation supplied animals for several experiments. An ongoing AALAS training program ensured the maintenance of a high percentage of certified staff. Currently 93% of animal care and diet-preparation staffs are AALAS-certified and two members of the animal care management group are Certified Managers of Animal Resources (CMAR).

Contribution to FDA's Strategic Goals

VSS contributes to the FDA Strategic Goals through its support of all animal care-services that support the animal-based research projects in the various research divisions and as Principal Investigators and Co-Investigators on research projects. The VSS plays a critical support-services role in NCTR's biomedical research program. VSS personnel interact with individuals from every research division on a daily basis, providing expertise in animal care, diet preparation, laboratory animal medicine, and microbiology. These services are provided by highly trained, skilled, and dedicated individuals whose contributions enhance the quality of the research conducted by NCTR scientists.

Cooperative Research and Development Agreements

NCTR actively pursues and maintains partnerships with nongovernmental organizations, nonprofit organizations, and private companies through Cooperative Research and Development Agreements (CRADAs). The FY 2014/2015 CRADAs supporting NCTR research projects include those listed below.

Toxicology Excellence for Risk Assessment (TERA)

Addendum: Development of a Method To Use *In vivo* Mutagenicity Data to Address the Question as to Whether a Specific Chemical Induces Cancer Via a Mutagenic or a Non-mutagenic Mode-of-Action (MOA) (E0722911)

University of Illinois

Addendum: Phytoestrogens and Aging: Dose, Timing, and Tissue (E0721021)

Women's Health Research at NCTR

In 2008, NCTR formed a Women's Health Research program within the Office of the Director and also formed a seminar series to promote and coordinate women's health research. The NCTR women's health mission is to foster research excellence regarding the influence of gender on the health of women, then to apply these research findings to address the health challenges and policies of FDA. The Women's Health Research Group is an interdivisional working group of scientists working on an active and innovative research program that focuses on understanding: 1) the molecular basis of drug efficacy and safety; 2) how genetic, epigenetic, sex/gender, diet, and other environmental factors influence drug efficacy and safety as it relates to women's health; and 3) computational framework for drug sensitivity. This group also coordinates women's health research projects funded by NCTR, FDA's Office of Women's Health (OWH), and extramural grants and partnerships to ensure the research fills knowledge gaps in the safety and efficacy of FDA-regulated products as they relate to gender differences in improving women's health.

In 2014, FDA's OWH Director of Research and Development continued to increase NCTR's visibility in its Women's Health Future Roadmap for Research. This visibility within their research model increases NCTR's collaborative leverage with other FDA Centers and increases NCTR's capacity in women's health-related research. NCTR's OWH Coordinator and liaisons continued to work with FDA OWH in finalizing their goals for FY 2015. A description of NCTR research capabilities was included in the 2014 OWH grant announcement. NCTR scientists working in several NCTR research divisions participated in scientific meetings, both nationally and internationally, and workshops sponsored by FDA's OWH, National Institutes of Health, the Society for Women's Health Research, and the Organization for the Study of Sex Differences. A critical focus of this participation was on developing successful strategies for engaging women and minorities in clinical trials and the importance of promoting interdisciplinary collaborative research to address the gaps in scientific knowledge about women's health.

In August 2014, NCTR hosted its Annual Women's Health Research Day that focused on the theme, "Moving into the Future with New Strategies to Advance Women's Health." Two keynote speakers presented research in cardiac induced pluripotent stem cells for disease modeling and drug discovery and new therapeutic targets and drugs for autoimmune diseases. FDA's OWH updated NCTR on its new vision for FDA Regulatory Impact in a report entitled, Future Roadmap for Women's Health Research, given by FDA's Deputy Director and Senior Medical Officer. In addition, the 2015 Goals and Objectives for OWH intramural leveraging funding were presented. For the second year, scientists at NCTR were given an opportunity to present potential funding ideas for critique and input from FDA's OWH. Large disparities remain in women's health and mortality rates among certain ethnic groups in breast cancer, autoimmune diseases,

cardiovascular disease, and cervical cancer. These disparities continue to increase despite major advances in overall survival outcomes for these diseases.

NCTR 2014 OWH awardees' accomplishments are listed below:

- Identified common and ethnic population-specific breast-cancer markers. A procedure that combined segmentation, clustering, and a cut-off estimate to automatically estimate a copy number change that was developed for breast cancer.

    1. Demonstrated that ductal carcinomas have measureable levels (>10-5) of PIK3CA H1047R mutation.
    2. PIK3CA H1047R exists as mutant subpopulations in ductal carcinomas meaning they have levels of PIK3CA H1047R mutation that would go undetected by DNA sequencing
    3. PIK3CA H1047R MF measurements in TNBC are significantly lower than that measured in normal breast. Additionally, showed a significant increase in the BRAF codon 600 GAG mutation, as compared to that measured in normal breast.

- Probabilistic model development and analysis were successfully conducted using Monte-Carlo methods and cluster computation applications. The resultant probabilistic model predictions provide a good representation of the maternal thyroid hormone levels and urinary iodide levels observed in the pregnant population of the United States and worldwide.

- Developed an *in vitro* model for high-throughput screening and risk assessment of torsadogenic drugs using a hormone-free medium and a serum-free medium for iCells from CDI and Axiogenesis. Further demonstrated that Dofetilide-induced proarrhythmia can be detected in both types of cells.

- Demonstrated that FOXP3, a member of the forkhead family of transcription factors and plays an important role in genes involved in regulatory T-cell (Tregs) function, is significantly downregulated in Systemic lupus erythematosus (SLE) through promoter methylation.

- Confirmed methodology that evaluates the effects of potential drug-delivery nanomaterials on *Candida albicans* infection of vaginal epithelial cells.

- Identified epigenetics biomarkers in SLE.

- Demonstrated three new targets, FOXP3, TNFSF13B (BAFF), and IL-18 in SLE as new targets for emerging therapeutics.

NCTR Projects Supported by OWH in FY14 or FY15

Blood pressure threshold for cardiovascular disease risk: an assessment of sex-based criterion (E0754201)

Clinical and Biological Significance of Three Identified Targets in Systemic Lupus Erythematosus Patients PBMCs: IL-18, TNFSF13B and FOXP3-Pilot Study (E0744611)

Development of Methods for Determining Nanoparticle Penetration/Permeation into Vaginal Mucosal Tissue (E0750701)

Evaluation of Methods Used to Measure Growth of Staphylococcus aureus and the Production of Toxic Shock Syndrome Toxin-1 as Influenced by Menstrual Tampons

Identifying Drugs that Cause Female-Biased Hepatotoxicity by Analyzing FDA Drug-Approval Packages/Labels and FDA-Maintained Databases and Conducting Comparative Studies in Primary Hepatocytes of Rats, Mice, and Humans (E0750201)

Integrated Analysis Of Single Nucleotide Polymorphism And Copy Number Variation In Genome Association of Breast Cancer (E0744401)

Population-Based Computational Framework for Assessing Xenobiotic Disposition and Interaction Effects in Pregnant Women-Pilot Study (E0752201)

Sex differences in drug-induced QT prolongation and torsade de pointes: establishing an *in vitro* model for high-throughput screening and risk assessment of torsadogenic drugs E0754001

Office of Minority Health

FDA's Office of Minority Health (OMH) Program started in 2013 as required by the Affordable Care Act to support FDA's mission. OMH will also work to support the HHS Office of Minority Health's efforts, to eliminate racial and ethnic disparities, to improve minority health, and to improve the quality of health care that minorities receive.

Minorities are under-represented in clinical research and trials, particularly those in therapeutic areas, which affect minorities disproportionally, such as diabetes, cardiovascular disease, hypertension, stroke, AIDS, lupus and certain cancers, such as triple-negative breast, prostate and pancreatic cancers. NCTR scientists are currently conducting research on triple-negative breast cancer using oncomutation profiles and epigenetic regulation of specific genes. Triple-negative breast cancer mortality rates are disproportionally higher in African-American women. Scientists are conducting research on pancreatic cancer which affects African-Americans at a higher rate. Pancreatic cancer has poor prognosis and a high mortality rate. Scientists at NCTR in 2014 showed that polymorphisms in drug transporters, the ABC family could play a role in chemoresistance and etiology of the disease. NCTR scientists are conducting extensive research on biomarkers and potential new epigenetic therapeutic targets in lupus. Lupus is known to affect African-American and Hispanic women at higher rates. In addition to research, NCTR scientists in 2014 worked with organizations in the community through health fairs and seminars in educating the public on diseases affecting their communities at alarming rates. Moving forward, NCTR will promote and coordinate research studies within NCTR to improve minority health.

In FY 2014 NCTR began a project that measures potential ethnic differences in inflammatory biomarkers in those with Type 2 diabetes and Alzheimer's Disease. Post-mortem tissue (brain, serum, adipose, liver) will be obtained from African-Americans and Caucasians with Type 2 diabetes and Alzheimer's Disease. The results of the project could explain the increased severity of symptoms in African-Americans comorbidity for these two diseases. Such results may enable better personalized medicine for those affected by these devastating diseases.

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Office of the Associate Director for Regulatory Activities (ADRA)

Donna L. Mendrick, Ph.D., Director
301-796-8892
Donna.Mendrick@fda.hhs.gov

Introduction

This office oversees NCTR's Science Advisory Board and acts in a scientific liaison capacity between NCTR and FDA's Regulatory Centers.

FY 2015 Plans

In 2015 we will continue to augment our interactions with FDA's Regulator Centers to help them with data gaps.

Division of Biochemical Toxicology Summary of Activities

Frederick A. Beland, Ph.D., Director
870-543-7205
Frederick.Beland@fda.hhs.gov

Introduction

The Division of Biochemical Toxicology conducts fundamental and applied research designed specifically to define the biological mechanisms of action underlying the toxicity of products regulated by, or of interest to, FDA. This research centers on quantifying the toxicities and carcinogenic risks associated with specific chemicals and introducing new risk-assessment techniques to enable regulatory agencies to better evaluate the risks associated with exposure to chemicals. The risk-assessment research is firmly rooted in mechanistic and exposure assessment studies focused on the understanding of toxicological endpoints, an approach that allows greater confidence in subsequent risk assessments. Research within the Division capitalizes on scientific knowledge in the areas of biochemistry, organic and analytical chemistry, cellular and molecular biology, nutritional biochemistry, toxicology, phototoxicology, computational risk-assessment methods, and pharmacology. Division investigators work in close collaboration with scientists in FDA Product Centers, the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP), and academia to address FDA's regulatory needs.

FY 2014 Accomplishments

A major theme within the Division continues to be toxicological assessments on compounds nominated by FDA for evaluation by NIEHS/NTP. This focus reflects NCTR's superb animal facilities supported by a multidisciplinary staff of scientists with strong mechanistic-research experience, which allows sub-chronic and chronic toxicological assessments to be conducted in a rigorous manner, often in compliance with FDA's Good Laboratory Practice guidelines. These studies currently serve as the benchmark by which toxicological assessments are made by FDA, other federal agencies, and international regulatory bodies. In addition to providing basic information on toxicological endpoints, such as cancer, these experiments form the basis for mechanistic and exposure-assessment studies to ascertain whether or not the response detected in the experimental model is pertinent to humans.

A principal area of research within the Division is the assessment of toxicities associated with exposures to dietary contaminants and dietary supplements. During FY 2014, Division investigators completed an assessment of the carcinogenicity of glycidamide, a

metabolite of acrylamide, a water-soluble α,β-unsaturated amide that is produced in the baking and frying of starchy foods, including French fries, potato chips, and bread. The results, combined with data from previous mechanistic studies, provide strong support for the concept that acrylamide is activated to a carcinogen through metabolism to glycidamide. These findings will allow the FDA's Center for Food Safety and Applied Nutrition (CFSAN), which nominated acrylamide to NIEHS/NTP for a toxicological assessment, to establish the risk of dietary exposures to acrylamide.
In further investigations of foodborne carcinogens and at the request of CFSAN, experiments continued to characterize the risks associated with exposure to furan. Furan is another dietary contaminant produced during the cooking of many common foods, including coffee, baked or fried cereal products, canned and jarred foods, baby food, and infant formula. The carcinogenicity of furan has been assessed in mice and rats; however, the risk to humans from dietary exposure to furan cannot be estimated because the lowest dose of furan tested in rats resulted in nearly a 100% tumor incidence. With funding from NIEHS/NTP, a reassessment of the carcinogenicity of furan is being conducted. In addition to conducting the bioassay, mechanistic studies are being performed with the goal of providing a solid foundation to establish the risks to humans from dietary exposure to furan. During FY 2014, a draft pathology report was prepared and reviewed by a pathology working group. In addition, a paper was published describing the dose- and time-dependent epigenetic changes in the livers of rats exposed to furan.

An additional area of investigation within the Division is the elucidation of potential toxicities associated with dietary exposures to putative endocrine-disrupting chemicals. Much of this emphasis has been placed on bisphenol A, to which there is ubiquitous exposure from food products and other environmental sources. This research effort, which is supported by CFSAN and FDA's Center for Devices and Radiological Health (CDRH) and funded by NIEHS/NTP, aims to address conflicting data in the literature regarding non-monotonic dose-response effects of bisphenol A at doses below the currently accepted no-observed-adverse-effect level. During FY 2014, Division investigators completed a 90-day subchronic study and comprehensive pharmacokinetic evaluations of bisphenol A. The results indicate that only doses much higher than estimated human exposures induced adverse effects. Division scientists also constructed a physiologically based pharmacokinetic model for young and adult rats to explore the species differences in age-dependent bisphenol A pharmacokinetics and to compare the model-predicted dosimetry of bisphenol A in rats, monkeys, and humans. On-going experiments include a two-year chronic toxicity assessment of bisphenol A. As part of this bioassay, tissues have been provided to 13 NIEHS academic grantees, who are examining additional endpoints that have been reported to be associated with exposure to bisphenol A.

During FY 2014, Division investigators completed and published a toxicokinetic study of triclosan, a broad-spectrum antimicrobial agent present in a wide variety of antibacterial soaps, deodorants, toothpastes, cosmetics, fabrics, plastics, and other products, which

was nominated by the FDA's Center for Drug Evaluation and Research (CDER) to NIEHS/NTP. In addition, a manuscript describing a 13-week sub-chronic dermal study was submitted for publication. Based upon the data obtained in the toxicokinetic and sub-chronic studies, a 2-year dermal carcinogenicity bioassay was initiated.
In the event of a bioterrorism attack on a food-production facility, chemical decontamination methods will be needed that have been tested and proven to be effective. With support from the National Center for Food Protection and Defense, Division investigators, in collaboration with scientists at CFSAN and the Institute for Food Safety and Health, performed experiments to evaluate the efficacy of different pasteurization conditions for inactivating ricin, a potential bioterrorism agent, in milk using commercially available pilot-scale pasteurizing equipment.

Adjuvants are an important component of vaccines because they allow reduced amounts of antigen to be used to achieve an acceptable level of immunological protection. In the case of pandemic influenza or the use of select agents in a biological attack, the ability to use lesser amounts of antigen per administered dose would be critical in protecting as large a population as possible. Some adjuvant-antigen vaccines, however, have been associated with adverse events that may be related to adjuvant immuno-stimulation. As part of an effort to model immunological mechanisms of efficacy and safety, Division investigators, in collaboration with scientists at FDA's Center for Biologics Evaluation and Research (CBER), acquired pharmacokinetic data on adjuvants containing α-tocopherol.

Pyrrolizidine alkaloid-containing plants are widespread in the world and are probably the most common poisonous plants affecting livestock, wildlife, and humans. During FY 2014, Division investigators characterized adducts resulting from reactions of pyrrolizidine metabolites with proteins. They also demonstrated that glutathione conjugates of pyrrolizidine alkaloids are electrophilic in nature and can react with DNA.
Skin-care products containing vitamin A congeners are among the most widely used agents for the mitigation of fine wrinkles, hyperpigmentation, and tactile roughness of photo-damaged and chronologically aged skin. Retinyl palmitate is the major storage form of vitamin A in the skin and is commonly incorporated into cosmetic creams and lotions. Retinyl palmitate absorbs ultraviolet light from sunlight, which could result in phototoxicities. At the request of CFSAN and with funding from NIEHS/NTP, the in-life phase of a one-year photo-co-carcinogenicity study of retinyl palmitate was completed during FY 2014.

A strong emphasis within the Division continues to be determining whether epigenetic changes (*e.g.*, DNA methylation) induced by carcinogens and found in tumors play a causative role in carcinogenesis or are merely a consequence of the transformed state. As part of these investigations, Division scientists have assessed the potential role of epigenetic changes as early markers of carcinogenicity. They have demonstrated that the tumor response in a mouse model of chemically induced fibrosis-associated liver carcinogenesis was associated with marked epigenetic changes rather than mutations in

known cancer-related genes. These findings suggest that the assessment of carcinogen-induced epigenetic alterations, in addition to genetic changes, may substantially improve the safety evaluation of products of interest to FDA and facilitate novel approaches for identification of subpopulations susceptible to exposures. Division investigators also have shown that epigenetic alterations may be a hallmark in autoimmune diseases, such as systemic lupus erythematosus (SLE). Specifically, changes in DNA methyltransferases, specific promoter methylation of critical genes in innate immunity, and modulation of specific microRNAs were identified in blood samples from SLE patients.

During FY 2014, Division investigators, with funding from FDA's Center for Tobacco Products (CTP), continued experiments in animals to assess the toxicity associated with exposure to smokeless tobacco products. The endpoints being examined include the characterization of DNA adducts and metabolites arising from tobacco-specific nitrosamines, as well as molecular changes resulting from these exposures. Division scientists also established an analytical chemistry laboratory designed to support various inhalation exposure studies and other CTP-sponsored tobacco-related experiments. A liquid chromatography-tandem mass spectrometer system and associated equipment were set up and utilized to verify the exposure concentrations for a pharmacokinetic study with nicotine-derived nitrosamine ketone (NNK), a tobacco-specific nitrosamine. Division investigators, in collaboration with CDER scientists, also continued experiments with oseltamivir (Tamiflu) in support of a study sponsored by the FDA's Medical Countermeasures Initiative.

FY 2015 Plans

In FY 2015, Division of Biochemical Toxicology investigators will:

- Prepare a draft report on a chronic bioassay of the food contaminant furan.
- Prepare a draft report on the toxicities associated with a sub-chronic exposure to silver nanoparticles.
- Prepare draft reports on 13-week and one-year photo-co-carcinogenicity studies on retinyl palmitate.
- Prepare a draft report on the sub-chronic study to evaluate the toxicities of melamine in combination with cyanuric acid in adult rats.
- Continue the two-year chronic bioassay to characterize the toxicities of bisphenol A in rodent models, with special emphasis on perinatal exposures.
- Continue pharmacokinetic investigations of bisphenol A in human subjects.
- Continue a 2-year chronic study to investigate the toxicities of topically applied triclosan.

- Continue to investigate the potential of pyrrolizidine alkaloid-protein adducts to serve as biomarkers of pyrrolizidine alkaloid exposure.
- Continue to develop methods for the rapid detection of potential bioterrorism agents in foods.
- Continue to investigate the role of epigenetic and microRNA alterations as potential biomarkers for noninvasive evaluation of exposure to genotoxic and non-genotoxic compounds of interest to the FDA.
- Continue to investigate the pharmacokinetics of the tobacco-specific nitrosamine NNK via oral and inhalation routes of exposure.
- Continue studies to evaluate the correction of epigenetic abnormalities as a novel approach for personalized cancer prevention.
- Continue investigations on the pharmacokinetics of vaccine adjuvants containing squalene and α-tocopherol.
- Continue studies to investigate the induction of arrhythmia in induced human pluripotent stem cell-derived cardiomyocytes.
- Continue to develop a physiologically based pharmacokinetic model to describe the kinetic behavior of bisphenol A in humans.
- Using computational approaches, investigate the effects of thyroid active chemical mixtures on thyroid hormone homeostasis in pregnant women and their fetuses.
- Using computational approaches, investigate the utility of animal models in determining the effects of pregnancy on the pharmacokinetics of oseltamivir (Tamiflu) in humans.
- Initiate studies on the time- and dose- response relationship of miRNA dysregulation in radiation-induced heart disease (RIHD), and the potential of using circulating miRNAs as biomarkers for early diagnosis and monitoring of RIHD.
- Initiate photo-co-carcinogenicity studies on the diuretic hydrochlorothiazide.
- Initiate sub-chronic studies with *Aloe vera* whole leaf preparation components, specifically aloin.
- Initiate studies on brominated vegetable oil, an additive used in the food industry to stabilize emulsions of citrus oils in beverages.
- Initiate studies on nattokinase and lumbrokinase, which are naturally-occurring fibrinolytic enzymes that are promoted as health supplements for human use.
- Initiate pharmacokinetic studies to develop physiologically based pharmacokinetic models for nicotine across different species.
- Initiate investigations into herb-drug interactions using physiologically based pharmacokinetic modeling.

- Initiate studies to determine the reversibility of the nephrotoxicity elicited by combined exposures to melamine and cyanuric acid.

Contributions to FDA's Strategic Priorities/Goals

The research conducted by the Division of Biochemical Toxicology contributes to FDA Strategic Objective 1.1 (*Increase the Use of Regulatory Science to Inform Standards Development, Analysis, and Decision-making*) and 1.3 (*Strengthen Detection and Surveillance of Problems with FDA-regulated products)*:

A major emphasis of the Division's research is to ensure the safety of food products, this supports FDA Strategic Objective 1.3. This is accomplished in close coordination with CFSAN and other FDA product Centers, which identify research needs and data gaps that guide the design of the Division's studies. For example, Division investigators have conducted bioassays and mechanistic studies to assess the risk of dietary exposures to acrylamide, a known rodent carcinogen and neurotoxicant that has been identified in coffee and baked and fried starchy foods—notably French fries, potato chips, and bread. A similar research strategy was applied to furan, another contaminant in food. Evaluations are also being conducted on bisphenol A, a chemical derived primarily from food-contact uses to which there is ubiquitous environmental exposure, and on *Aloe vera*, a natural product incorporated into dietary supplements. As part of the Division's efforts to ensure the safety of foods, assays are being developed and applied to detect the biological activities of potential bioterrorism agents, for example ricin and abrin, in various food products. Division investigators are also conducting studies to assess the toxicities associated with exposure to melamine, cyanuric acid, and pyrrolizidine alkaloids, contaminants that have been found in certain food products.

Computational tools are being developed within the Division to integrate toxicological, mechanistic, and pharmacokinetic data for safety assessments, supporting FDA Strategic Objective 1.1. Physiologically based pharmacokinetic models of bisphenol A in rats, non-human primates and humans have been developed to reduce the uncertainty in predicting human health risks from exposure to bisphenol A. Computational analyses have been employed for the assessment of pharmacokinetics and interspecies extrapolation across non-human primates and humans for toxicities associated with methylphenidate. The results suggest that continued pharmacovigilance is prudent to monitor the safe use of this drug. Division scientists developed a computational model to evaluate the effects of perchlorate exposure and dietary iodide status on the hypothalamic-pituitary-thyroid axis of pregnant women and their fetuses. Model simulations indicate that environmentally relevant perchlorate exposure levels are far lower than the levels required to cause hypothyroxinemia in a typical pregnant woman. Division investigators have developed a highly sensitive, precise, and accurate liquid chromatography-isotope dilution tandem mass spectrometry methodology for the

quantification of the antiviral drug oseltamivir (Tamiflu) and its carboxylic acid metabolite in non-human primate serum in support of a protocol sponsored by FDA's Medical Countermeasures Initiative.

Division of Bioinformatics and Biostatistics Summary of Activities

Weida Tong, Ph.D., Director
870-543-7142
Weida.Tong@fda.hhs.gov

Introduction

The Division of Bioinformatics and Biostatistics (DBB) develops integrated bioinformatics and biostatistics capabilities to address demands in biomarker development, drug safety, drug repositioning, personalized medicine, and risk assessment. Its capability is directed towards integration with FDA business processes to ensure NCTR linkages with FDA Product Centers are strengthened, and that NCTR informatics capabilities continue to evolve to be capable of meeting future requirements of the FDA.

The division is comprised of three branches:

- Bioinformatics
- Biostatistics
- Scientific Computing

The **Bioinformatics Branch** conducts bioinformatics and chemoinformatics research in the fields of predictive toxicology, personalized medicine, biomarker development, drug safety, and drug repositioning. Most research projects of this group are in collaboration with scientists within NCTR, across FDA Product Centers, and in the larger scientific community. A goal of this group is to develop methods and standards for the analysis and integration of diverse data derived from various technologies including emerging genomic methods (such as next-generation sequencing and microarrays), classical in-life parameters and public data sources. One of the key endeavors of this group is to construct knowledge bases in the specific areas of FDA's responsibility to provide a data-driven decision-making environment for enhanced safety evaluation and personalized medicine. In addition, this group is taking an active role in supporting bioinformatics needs of other FDA Centers.

The **Biostatistics Branch** conducts peer-reviewed research and provides statistical support related to FDA's mission to protect and promote public health. The research statisticians develop new/improved statistical methods for risk/safety assessments aimed at FDA's goal of improving product safety and efficacy. A team of statisticians is dedicated to providing statistical support to the National Toxicology Program (NTP)-funded studies and NCTR scientists regarding the design, conduct, analysis, and

interpretation of results of studies for safety and efficacy of regulated products.

The **Scientific Computing Branch** provides critical support and enhancement to the infrastructure in the areas of software and database development for research support and research management; high-performance computing; systems integration; and information-system asset management and procurement.

FY 2014 Accomplishments

Bioinformatics Branch

- The amount of data present in the public domain and generated at NCTR is very difficult to interpret manually. Thus, bioinformatics approaches are being applied to identify connections that can be used for hypothesis-based research and to offer a new venue towards data-driven decision-making systems. One such project is the development of a Liver Toxicity Knowledge Base (LTKB). The goal of the project is to collect all clinical parameters of each drug that has been reported to cause liver injury. Then that information would drive identification of potential biomarkers that can be easily and cheaply obtained from preclinical models including *in vitro* studies, toxicogenomics, and *in silico* parameters. In 2014, an enhanced version of the LTKB database was developed, and made publicly available. The new version contains a large number of data for drugs along with the predictive models that can be accessed for prediction online. Most of the accomplishments in this project were published in peer-reviewed journals with high impact factors. This work continues.

- Another project involves leading an international consortium, called MicroArray Quality Control (MAQC) that assesses the reliability and practical utility of emerging technologies. In 2014, the consortium completed the third phase of MAQC, also known as Sequencing Quality Control (SEQC) project. The project assessed current next-generation sequencing technologies. This next-generation sequencing approach generates very large datasets that challenge analysis approaches. This consortium is looking at appropriate approaches and standards to enable expanded use of this developing science. The consortium published eight manuscripts, of which three manuscripts were in *Nature Biotechnology*, two were in *Nature Communication*, and three were in *Scientific Data*. There are three other manuscripts (one in *Nature Method* and two in *Genome Biology*) in the review stage. An upcoming special issue by the Nature Publishing Group will contain the full results of SEQC in eight manuscripts. The SEQC was an international effort of over 150 researchers from 12 countries and was coordinated by the FDA.

- Supporting the analysis of gene-expression results has been a standard function of the bioinformatics group. The group developed a software package that is used within and

outside FDA called ArrayTrack™. Improvements are being made to ArrayTrack™ to assist FDA studies on genomics data.

- This group continues to support other FDA Centers' needs that includes developing databases for their use and providing the subject-matter expertise to engage various bioinformatics efforts at FDA. One of these efforts is to develop a database for FDA drug labels. The drug labels represent the consensus and combined experience of regulators, drug sponsors and manufacturers, and scientific experts with information about product indications, target populations, and adverse drug reactions (ADRs) collected during clinical trials and post-marketing surveillance. The labels not only catalog safety and efficacy data related to population-wide treatment responses to drugs but also contain the responses pertaining to sex, age, race, and ethnicity. This group has developed FDALabel that contains the full set of approximately 60,000 FDA-approved drug labels. Version 2 of FDALabel has been developed, which is a web-application with functions of managing, querying, and organizing drug-label information. The tool has a user-friendly interface with searches against the entire text of drug labels and is implemented with a secure three-tier platform with an Oracle database as a server. The database is acquired and used by FDA reviewers on a small scale.

Biostatistics Branch

The Biostatistics research efforts focused on statistical methods to analyze toxicological and molecular data, and to develop/apply data-mining methods for pattern identification and signal detection of high-dimensional data. Branch scientists conduct both individual research within the division and collaborative research with scientists from other NCTR divisions, and other FDA centers. Major research accomplishments include the following:

- Biostatistical Methods – Statistical models/procedures have been developed to analyze microarray gene expression, single nucleotide polymorphism, copy number variation, and next-generation sequencing data for improved evaluation of safety/efficacy of FDA-regulated products. Algorithms are developed to identify individual genes and biological pathways associated with an individual's response to a treatment. Survival-risk prediction models have been developed and investigated for cancer patients for treatment selection. Statistical procedures are being developed for QT analysis and cardiotoxicity, gender difference in blood pressure threshold, and categorization of uncertainty in risk/safety assessment.

- Data Mining Techniques – Clustering, biclustering, and classification algorithms have been developed for subgroup identification and prediction. These techniques have been applied to the development of prognostic, predictive models, and predictive enrichment classifiers in personalized medicine, serotype identification and characterization in outbreak investigation, and identification of drug subgroup to

adverse-event subgroup association in the FDA Adverse Event Reporting System database.

- Foodborne Pathogens *Salmonella* Genomics Knowledge base – By using the 45,923 pulsed-field gel electrophoresis (PFGE) data stored in the BACPAK knowledge base, statistical methods were developed to perform analysis of the PFGE patterns, and revealed for the first time the close relationships and similar PFGE patterns of some serotypes of *Salmonella,* and distinguished the top 10 most frequent bands for each of the 32 serotypes. The reference fingerprint pattern for each serotype was also proposed. This work expanded the current knowledge of the diversity of PFGE patterns for various *Salmonella* serotypes, and contributed to better understanding of the performance and application of PFGE fingerprinting. This allows for effective source tracking and distinguishing *Salmonella* isolates related to foodborne outbreaks and clinical investigations.

- Novel methodologies development on Next Generation Sequencing (NGS) data analysis of bacterial pathogen. A bioinformatics pipeline was developed and implemented for sequence acquisition and genetic diversity analysis from NGS data. The developed pipeline provides an effective bioinformatics tool for genetic diversity clarification and marker sequences discovery which will enhance the NGS data analysis and its applications on pathogen identification, source tracking, and population genome evolution.

- Computational software to compute p-values and adjusted p-values for gene-set enrichment analysis defined through pathways and gene ontology.

- Benchmark Dose Calculation for Ordered Categorical Responses – The use of benchmark dose (BMD) calculations for dichotomous or continuous responses is well established in the risk assessment of cancer and non-cancer endpoints. A collaborative project has developed a categorical regression model to extend the BMD approach to ordered categorical responses by modeling severity levels as censored interval limits of a standard normal distribution.

The Biostatistics Support Team has continued to support National Toxicology Program (NTP)-funded studies, including furan, nanosilver, melamine/cyanuric acid, oxybenzone, BHT/IPA, bisphenol A, and triclosan. In addition to providing support for NTP-funded studies, the Support Team performs statistical protocol review for all proposed NCTR studies, and conducts IACUC study reviews for animal studies, statistical analysis, and support for non-NTP funded studies of methyphenidate, MPTP-probenecid, and tobacco constituents.

Scientific Computing Branch

The Scientific Computing Branch was responsible for supporting the software and database needs of the NCTR research and management staff.

- Protocol Data Collection System (PRODACS) – Continued to work with Office of Scientific Coordination staff and animal room staff to locate problems and tune the data collection component in preparation for testing and validation.

- Supported the Multispecies Behavior System hardware and software and developed units for offsite deployment as part of NCTR's Division of Neurotoxicology's collaboration with Mt. Sinai Hospital.

- Developed a new Primate System at the request of Dr. Merle Paule, Division Director of Neurotoxicology.

- Contributed to a manuscript for the Division of Systems Biology and an abstract for the Division of Neurotoxicology.

- Continued to modify the Electron Microscope Electronic Notebook (EMEN2) to support the needs of the NCTR NanoCore. This included implementing support for Personal Identity Verification card access in order to address security findings.

- Maintained the production and development of Oracle and Adabas databases. Led the migration of all Oracle Forms and Reports applications to a new version of the database environment including the Oracle Identity Management server, Oracle Access Manager and the WebLogic tier.

- Installed the latest version of Application Express (ApEx) and deployed multiple applications to the production and development environments.

- Designed a server and database platform for the latest version of SAS Activity Based Management.

- Developed a prototype for a new version of the Document Tracking application at the request of the Office of Research.

- Developed a new version of the NCTR Stockstore application (aka, INVM).

- Developed a new photox dosimetry application to replace the existing software that is not compatible with Windows 7. This application required GLP validation.

- Reformatted Multigen data in the Standard for the Exchange of Nonclinical Data and supported the loading of the data into the Chemical Effects in Biological Systems database at NIEHS.

- Modified Concept Tracking to support new requirements as requested by the Deputy Director for Research and Office of Management staff.

- Represented the Center on a number of Agency level committees to help ensure the needs of the research and support staff are met (including CIO Council, Data Standards Advisory Board, IT Liaisons, Change Control Board, IT Asset Management workgroup).

- Completed the Master Infrastructure Qualification Plan in cooperation with FDA's Office of Information Management Technology/Division of Infrastructure Operations and NCTR's Office of Scientific Coordination staff. This will guide the qualification of network and server hardware.

- Conducted meetings with administrators, faculty, and students from the University of Arkansas at Pine Bluff to establish avenues of collaboration and possible hiring of interns and summer students.

- Continued to pursue membership in the Arkansas Research and Education Optical Network (ARE-ON). This included distributing the results of a feasibility study and discussing with senior OIMT staff.

FY 2015 Plans

In FY 2015, the Division of Bioinformatics and Biostatistics will continue to emphasize a unified approach for development of safety and efficacy assessments of medical products and foods. New studies will begin to discover biomarkers of tobacco-related injury. To accomplish its mission, the Division of Bioinformatics and Biostatistics will continue with the efforts to:

- Expand the Liver Toxicity Knowledge Base in the following three aspects:
    - Integrate analysis of diverse data that represent a broad range of biological complexity.
    - Integrate analysis of different predictive models for an enhanced performance of predictive modeling.
    - Conduct additional experimentation using next-generation sequencing for identifying better translational biomarkers for drug-induced liver injury in humans.
    - Conduct study with *in vitro* assays to assess drug-induced liver injury.

- Expand the functionality of FDALabel with specific query mechanisms along with the implementation of standards. The database, once matured, will enhance FDA's capability to answer Congressional inquiries and consumer requests. The database will

also improve FDA's mission ofr protecting the public health including the health of women and demographic subgroups.

- Provide technical expertise to support the FDA review process. This effort includes upsizing the Data Analysis and Search Host program which is a key resource in FDA's Center for Drug and Evaluation Research to capture the review information.

- Build on the success of the 3rd phase of the MicroArray Quality Control (MAQC) project, also known as Sequencing Quality Control (SEQC), and leverage the SEQC data. New protocols will be developed to further expand the scope of SEQC to evaluate the technical performance and practical utility of next-generation sequencing (NGS) technology.

- Develop decision models for clinical assignments of patients based on the patient's genomic features and disease phenotypes. Experimental designs and data-analysis strategies for evaluation of studies for drug-device co-developed products will be developed.

- Evaluate blood pressure threshold for cardiovascular-disease risk to assess potential sex-based criterion.

- Develop methods to assess cardiac safety of drugs. Statistical modeling and procedures are being developed to obtain accurate measurements of QT interval correction to identify true QT prolongation.

- Develop bioinformatics methodologies and tools for NGS data analysis, data mining and visualization to fully achieve the benefit of NGS technologies on public health, especially on microbial pathogen detection and surveillance. These tools can be used to discover the robust predictive biomarkers for rapid detection and diagnostic tests including bacterial serotype identification, virulence determination, and antimicrobial-resistance tracking.

- Develop a novel data-mining method by applying topic modeling and other machine learning algorithms to the FDA's Substance Registration Systems databases for classification of products and for detecting adverse event safety signals. The proposed approach may potentially reduce the human workload, enhance the regulatory product review, and detect the unrecognized hidden adverse effects and the associations with the regulated products.

- Analyze data from National Toxicology Program studies.

- Complete development of PRODACS data collection and error correction components and begin the validation process in cooperation with Office of Scientific Coordination (OSC) staff.

- Under the governance of the Center IT Board, continue evaluation of legacy applications and prioritize the order in which the applications are addressed. The results will be a streamlined portfolio of applications that are useful, compatible with current IT infrastructure, and compliant with FDA/HHS/OMB information system requirements.

- Many of the applications currently written in Oracle Forms and Reports will be converted to Oracle Business Intelligence and/or Application Express. These new applications will be more user friendly, more responsive, and compatible with updated infrastructure (e.g., newer operating systems and mobile platforms).

- Complete the development of a new Special Employment System (SEPS) and a new application to replace WinLIMS which is no longer supported by the vendor.

- Complete the development of the new Instrument Repair Work Order application (ISSWOR) per a request from the Contracting Officer of the Bionetics contract.

- Replace MBS Droids in the rat and non-human primate labs to ensure stability and compatibility with new operating systems and feature requests.

- Continue to support EMEN2 for the NCTR NanoCore and expand to support other Offices and Divisions as requested.

- Implement new pre-production and test Oracle environments to allow for proper testing of patches, updates and other modifications to Oracle databases and applications.

- Create an Oracle 12c environment to begin testing compatibility with NCTR infrastructure and legacy applications .

- Update the Master Validation Plan in cooperation with OSC.

- Provide an example to support a GitHub repository for software development at NCTR and ensure all code is tracked and versioned appropriately in accordance with the HHS Enterprise Performance Life Cycle.

Contributions to FDA's Strategic Priorities/Goals

The research conducted by the Division of Bioinformatics and Biostatistics contributes to FDA's Strategic Objective 1.1, (*Increase the use of regulatory science to inform standards development, analysis, and decision-making)*, FDA Strategic Objective 2.1 (*Increase regulatory science capacity to effectively evaluate products*), and FDA Strategic Objective 2.3 (*Improve the predictability, consistency, transparency, and efficiency of the review process*).

The Division provides expert advice and innovative research to the other FDA Centers, thus contributing to FDA's mission of advancing public health. Research projects involve new and innovative technologies and approaches that support FDA's Product Centers. Innovation will continue to be a hallmark of this Division and will include the development of new integrated and knowledge base methods to enable discovery of new types of biomarkers, new methodologies for safety evaluation and monitoring, and new ways of interpreting and integrating the massive and diverse data. The continued development of new bioinformatics tools will allow reviewers to easily access information from both the private and public domain—thus enhancing the FDA review process. Novel computational models will continue to be developed that predict drug safety and efficacy. These new methods will increase the number of safe and effective medical products.

The Division's Biostatistics branch is helping to develop and refine preclinical and clinical trial designs, endpoints, and analysis methods. Scientists are:

1) Continuing to refine preclinical /clinical trial design and statistical methods of analysis to address issues such as, multiple endpoints, biomarker and subgroup identification, prediction algorithm, and patient selection.

2) Continuing to refine the use of modeling and simulation in preclinical/clinical trial design to enhance the effectiveness of studies.

3) Continuing development and refinement of tools and approaches for assessing benefit/risk.

Examples include developing decision models for clinical assignments of patients based on the patient's genomic features and disease phenotypes, analysis of a number of high-dimensional data sets, QT prolongation and cardiotoxicity studies, as well as the development of a knowledge base for rapid threat assessment of enteric foodborne pathogens.

The Scientific Computing Branch will support the efforts of the Center by developing and maintaining innovative software tools necessary to perform regulatory research. The

combination of technical skills, research experience, and institutional knowledge will enhance collaborations with other Centers and Agencies and allow for efficient management of NCTR's information system assets.

Division of Genetic and Molecular Toxicology Summary of Activities

Robert H. Heflich, Ph.D., Director
870-543-7493
Robert.Heflich@fda.hhs.gov

Introduction

The Division of Genetic and Molecular Toxicology (DGMT) conducts research to improve FDA's safety assessments of regulated products. The Division's goals are to promote public health by providing FDA with the expertise and tools necessary for comprehensive assessments of genetic risk and to strengthen approaches to integrate knowledge of genetic risk into regulatory decision-making. Division research is directed towards improving existing methods, and towards developing and validating new methods for evaluating high-priority issues related to the toxicity of food additives, human and animal drugs, biological therapies, nanomaterials, dietary supplements, tobacco products, botanicals, and medical devices. In collaboration with other FDA scientists, DGMT utilizes the methodologies it develops to better understand the potential toxicity of specific high-priority products of concern to FDA regulators.
As experts in the field of genetic toxicology, DGMT scientists maintain a leadership role in regulatory assay development and validation. Division scientists are actively involved in national and international efforts to harmonize genetic toxicology tests and to improve their interpretation and use for regulatory decision-making. Division scientists actively participate in the Organization for Economic Cooperation and Development (OECD) expert workgroups that are revising the current Genetic Toxicology Test Guidelines and currently are developing additional guidelines for genetic toxicology testing. Division scientists frequently provide expert advice to FDA Product Centers, other government agencies, academia, and industry. DGMT scientists also are active participants in the FDA GeneTox Network and interagency workgroups.

Research Themes:

1) Development, Validation, and Maintenance of Regulatory Genetic-Toxicology Assays

2) Chemical-Specific Research

3) Development of New Paradigms for Regulatory Decision-Making that Integrate Measures of Genetic Risk with Biomarkers of Toxicity

   - Develop More Relevant Biological Models
   - Develop More Comprehensive Approaches to Monitoring Genetic Variation
   - Develop Better Ways of Evaluating Data to Determine Human Risk

FY 2014 Accomplishments

DGMT scientists actively participate in FDA and international working groups that form consensus on how to conduct regulatory genetic toxicology testing. International working groups include those of the International Workshop for Genotoxicity Testing (IWGT), the OECD, and the International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI). Division scientists will continue to be involved in discussions concerning the appropriate strategies for conducting risk assessments of regulated products.

Specific FY 2014 research accomplishments involving developing, validating and maintaining standard regulatory assays include:

- Performed studies to develop a model for exposing suspension cultures of mammalian cells to whole smoke generated by a cigarette smoking machine.
- Served as a member of the FDA Taskforce for nanomaterials.
- Participated in OECD Working Groups for updating existing genetic toxicology Test Guidelines, and for developing guidance for the evaluation of nanomaterials.
- Contributed to a new OECD Test Guideline on the *in vivo* Comet assay by conducting research to evaluate important parameters of this technique.
- Led an IWGT Workgroup that produced a consensus document on using the *Pig-a* assay for regulatory safety assessments.
- Led an ILSI/HESI Working Group to develop the *in vivo Pig-a* gene mutation assay as a regulatory genotoxicity test.
- Conducted research evaluating the usefulness of standard *in vitro* assays for assessing the genotoxicity of tobacco products.
- Conducted RNA-seq and data analysis to identify transcripomic variations in human B-lymphoblastoid lines used in genetic toxicology testing.
- Published papers on using the mouse lymphoma assay, the *Pig-a*, *Hprt* and transgenic rodent *in vivo* gene mutation assays, chromosome painting, and the *in vitro* Comet assay for genotoxicity testing.

- In response to IWGT and ILSI/HESI recommendations, developed a flow cytometric sorting method to effectively characterize lymphocyte *Pig-a* mutations induced in the rat.

The Division employed standard genotoxicity assays to generate chemical-specific testing data that can be used by the FDA Product Centers, including:

- In collaboration with FDA's Center for Tobacco Products (CTP), conducted research with human in vitro airway cultures to assess the toxicity caused by chemicals present in cigarette smoke and by cigarette whole-smoke solutions.

- In collaboration with CTP, conducted research with standard assays to evaluate the genotoxicity caused by chemicals present in cigarette smoke and by cigarette whole-smoke solutions.

- In collaboration with CTP, conducted research on the genotoxicity of different extracts of smokeless tobacco in standard in vitro assays.

- In collaboration with an FDA Center for Drug Evaluation and Research (CDER) reviewer, published a study on the in vivo genotoxicity of a common drug impurity.

- At the request of FDA CDER reviewers, completed studies and published findings on the mutagenicity of two different impurities often seen in pharmaceuticals.

- At the request of an FDA Center for Food Safety and Nutrition (CFSAN) reviewer, completed research on the mutagenicity of Gingko biloba samples used in a National Toxicology Program study.

- In collaboration with CFSAN scientists, conducted research and published a paper on the in vivo genotoxicity of two common food additives—estragole and safrole.

- Published papers on the ability of the *in vivo Pig-a* gene mutation assay to detect the genotoxicity of a dietary supplement (aristolochic acids) and a series of highly cytotoxic agents used for cancer chemotherapy (X-rays, cyclophosphamide, cisplatin).

- Completed a collaborative study with CTP on DNA damage induced by a high-priority chemical from cigarette smoke in rats treated by different routes of exposure.

Progress was made in FY 2014 in developing new paradigms for regulatory decision-making that integrate measures of genetic risk with biomarkers of toxicity, including:

- Conducted research using an allele-specific competitive blocker-polymerase chain reaction (ACB-PCR) technology. Progress indicates that this approach provides the opportunity to detect rare mutations involved in the etiology of cancer prior to the

development of the actual visible tumor. ACB-PCR provides a strategy that might ultimately lead to replacing the traditional two-year cancer bioassay and hasten the development, safety assessment, and approval of new drugs.

- In collaboration with scientists at Harvard University, studied the relationship between hydroxylmethylation of cytosine in DNA and genetoxic carcinogens.

- Continued participation in an international trial of a new approach for analyzing gene mutations *in vivo*. This assay, co-developed by Division scientists, uses fluorescent probes to detect mutation in the endogenous *Pig-a* gene. The assay lends itself to sensitive, rapid, and minimally invasive mutation analyses in humans and animal models.

- Published article on the toxicity and efficacy of carbon nanotubes and graphene and the utility of carbon-based nanoparticles in nanomedicine.

- Published a research paper and a review article on the role of microRNA in modulating mutagenicity in genotoxicity assays.

- Conducted research on the development of a new method using microRNA expression as a biomarker for identifying carcinogens.

- Developed a new research protocol on the evaluation of microRNAs in blood and urine for the detection of chemical-induced carcinogenicity.

- In collaboration with CTP, developed methodology for performing histochemical analysis and measuring tight junction integrity, protein oxidation, extra-cellular matrix proteins, cilia beating, and mucus production as endpoints of tobacco-product exposure model in cultures of human-airway cells.

- Conducted in vivo genotoxicity studies on three agents as part of a large multi-organization project, funded by a Cooperative Research and Development Agreement (CRADA) with Toxicology Excellence for Risk Assessment (TERA), to evaluate the use of in vivo mutation data to inform cancer mode-of-action.

- As part of the TERA CRADA, published a dose-response ethylene oxide inhalation study that measured the temporal induction of oncogenic K-ras mutations in relation to lung tumorigenicity in mice.

- Published results from an in vivo transgenic-mouse mutation study on the effect of the background mutant frequency on the mutagenicity dose response for genotoxic carcinogens. This study, which employed computational dose-response modeling, revealed that the shape and magnitude of the dose-response can vary depending on the mutational endpoint, the tissue evaluated, and the mutational model used.

- Applied Next Generation Sequencing to evaluate DNA sequence changes in a pool of flow-sorted Pig-a mutants.

- Published an article indicating KRAS mutant subpopulations are present in a majority of human lung adenocarcinoma tumors and are drivers of resistance to α-EGFR therapy and patient relapse. Published a review indicating that mutant subpopulations in tumors may be a general problem for failure of personalized medicine cancer therapy.

FY 2015 Plans

Specific plans include:

- Complete a validation study on a tobacco-smoke generating machine and *in vitro* cell-exposure models recently acquired to perform CTP collaborative studies.

- Start a new CTP-sponsored project utilizing the *in vivo* Comet assay to study tobacco inhalation exposure studies in Sprague Dawley Rats.

- Continue an Office of Women's Health-funded project determining the oncomutation profile of triple-negative breast cancers.

- In collaboration with the University of Arkansas for Medical Sciences, begin a project aimed at developing a human reticulocyte *PIG-A* assay for use in cancer patients receiving platinum-based antineoplastic therapy.

- Continue a CTP-sponsored project measuring the toxicity and inflammation induced by whole smoke generated from a tobacco smoke-generating machine in human *in vitro* airway cultures.

- As part of the CORES initiative, determine whether the current genetic toxicology assays are appropriate for evaluating the potential toxicity of nanomaterials.

- Develop a positive control for nanomaterial genotoxicity assays.

- Continue RNA-seq data analysis and prepare a manuscript cataloging mutations identified in human B-lymphoblastoid lines used in genetic toxicology testing to submit for publication, as well as to continue evaluating the utility of Next Generation Sequencing for applications in genetic toxicology.

- Validate the rodent *Pig-a* gene mutation assay by developing a human reticulocyte *PIG-A* assay to evaluate the ability of the rodent *Pig-a* assay to predict the genotoxicity of FDA-regulated products in humans.

- Develop microRNA biomarkers for mutagens and carcinogens.

- Evaluate microRNAs in blood and urine for detection of chemical-induced carcinogenicity.

- As part of a Memorandum of Understanding between the State of Arkansas and FDA, perform research on the genotoxicity of the nanomaterial, graphene.

- In collaboration with CDER scientists, continue evaluating the hypothesis that rare oncogene mutations in human tumors are responsible for the failure of personalized medicine therapies by tracking mutation subpopulations *in vitro* in spheroid cultures generated from human-lung tumors.

- Conduct research investigating the background frequency of cancer mutations in rodent models. The study will include the potential impact of rodent strain and age.

- Investigate the use of Next Generation Sequencing to measure low frequency cancer-relevant mutations in normal and tumor tissue.

- In collaboration with TERA, use the model oxidant, vanadium pentoxide (VP) in a transgenic mutational model to investigate the mutagenic mode-of-action for the carcinogenicity of VP as well as relationships between oxidative stress, *Ras* activation, and carcinogenesis.

- Explore the ability of Next Generation Sequencing for detecting somatic cell mutation induction by mutagenic carcinogens. This methodology has the potential of determining mutational load in the entire genome, regardless of the phenotype of the interrogated sequence or the tissue source of the DNA.

- Test the utility of a transgenic hairless albino mouse mutational model (newly developed by Division scientists) for use in photogenotoxicity and photocarcinogenicity studies, and for assessing the safety of nanoparticles in cosmetics.

- Develop *in vitro* and *in vivo* methods to assess the global as well as gene-specific DNA methylation status using a single-cell gel electrophoresis assay.

- Evaluate the ability of a peripheral blood mononuclear cell system to act as an immunomodulator in a human *in vitro* assay for the immunotoxicity of nanomaterials.

Contributions to FDA's Strategic Priorities/Goals

The research conducted by the DGMT contributes to the following FDA Strategic Priorities, excerpted from the 2014-2018 Plan:

Goal 1 – Enhance Oversight of FDA-Regulated Products

- Objective 1.1: Increase the use of regulatory science to inform standards development, analysis, and decision-making:
    - advancing regulatory science by advancing the development of predictive safety models
    - translating new technologies into real-world diagnostics, treatment and cures

Goal 2 – Improve and Safeguard Access to FDA-Regulated Products to Benefit Health

- Objective 2.1: Increase regulatory science capacity to effectively evaluate products
    - Increasing collaboration and information sharing with colleagues in industry, academia, and other regulatory bodies
    - Supporting public private partnerships to advance regulatory science
    - Improving the efficiency and validity of safety evaluations of food ingredients and dietary supplements

Goal 4 – Strengthen Organizational Excellence and Accountability

Objective 4.1: Recruit, develop, retain, and strategically manage a world-class workforce

- Promoting cross-disciplinary regulatory science training, especially in the international arena
- Promoting opportunities for continuous learning and career development

The Division provides expert advice and innovative research to the FDA Product Centers, thus contributing to FDA's mission of advancing public health through improvements to regulatory science. Several research projects involve the development of new and innovative technologies and approaches that support safety reviews conducted by FDA's Product Centers. Other research explores approaches that may benefit the implementation of personalized medicine strategies.

Genetic toxicology is concerned with the ability of chemicals to alter genetic material. FDA currently requires that petitioners provide data evaluating the potential genetic toxicity of their products as a part of the product-approval process. Because evidence indicates that genetic damage is important in tumor development, this information is used in the evaluation of suspected carcinogens. Research within the Division focuses on the development and validation of new methods to assess genetic risk. Bacterial and tissue-culture approaches are commonly used to detect potential genotoxicity and to generate hypotheses concerning the basic mechanisms of genotoxicity.

Division scientists recently have begun developing and evaluating tissue-equivalent human *in vitro* models that may provide more relevant data for assessing human risk than traditional bacterial and rodent-based *in vitro* systems. Division scientists also specialize in the development and validation of new *in vivo* mammalian systems and the incorporation of these methods into risk-assessment strategies. An increased understanding of mutational mechanisms, combined with test systems that detect genetic damage in a manner relevant for assessing human risk, will provide FDA with better information for decision making. As new assays are validated, Division scientists will continue to work with international scientists to assure the harmonization of protocols and the development of guidelines to assess genetic hazards.

Genomic technologies are beginning to provide new tools for making better public-health decisions. The scientific and medical communities have benefited by an increased understanding of the genetic material and how it functions in both humans and rodents. Utilizing this information, new molecular technologies are being rapidly developed and can be used to evaluate structural and functional changes to the genetic material of both rodents and humans. The Division is using new technologies, in combination with more traditional approaches, to address various research questions.

While current technologies in the field of genetic toxicology generally evaluate single endpoints, newer approaches are providing the opportunity to detect alterations in a number of endpoints simultaneously. In the future, these new approaches will allow for the integration of information across the various types of adverse-health outcomes. For instance, when these technologies are fully developed, it will be possible to concurrently evaluate chemicals for their ability to cause cancer, to impact the nervous system, to cause birth defects, and to modify immune function, providing additional insight to the individual responses and increasing the efficiency and effectiveness of the regulatory process.

DGMT is committed to recruiting and retaining experts in the field of genetic toxicology, as well as providing continuing education training opportunities to current staff. Several staff members have completed a graduate certificate program at the University of Arkansas for Medical Sciences that was developed through a Memorandum of Understanding between the State of Arkansas and FDA. One staff member is currently attending a master's program in regulatory science at the University of Maryland Center Of Excellence in Regulatory Science and Innovation. This program was developed through a collaborative effort between FDA and University of Maryland, Baltimore and funded by a grant from FDA. DGMT also offers hand-on training opportunities through special employment opportunities, and currently has a number of Postdoctoral Fellows and an FDA Commissioner's Fellow. In the past year DGMT also has hosted scientists from Wales, China, and Mexico for short-term training opportunities.

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Division of Microbiology Summary of Activities

Carl E. Cerniglia, Ph.D., Director
870-543-7341
Carl.Cerniglia@fda.hhs.gov

Introduction

The Division of Microbiology goals are to perform fundamental and applied research to address critical issues in support of the FDA mission. The Division of Microbiology research projects are based on expertise of division staff and consultation with scientists from other FDA Centers, regulatory agencies, academia, and industry. During FY 2014, the Division of Microbiology scientists engaged in research addressing FDA issues with special emphasis on: 1) developing methods to detect, identify and characterize foodborne pathogens; 2) determining antimicrobial resistance and virulence mechanisms of microbial pathogens; 3) using state-of-the-art molecular biological approaches to monitor interactions between human microbiota, antimicrobial agents, food contaminants, food additives, food supplements, nanomaterials, and FDA-regulated products; 4) conducting studies impacting women's health; 5) improving environmental risk assessments of priority pollutants, including polycyclic aromatic hydrocarbons and drugs, by integrating systems biology approaches; 6) developing new models of smokeless tobacco toxicity with the Center for Tobacco Products; and 7) conducting nanotechnology research. Selected accomplishments by Division Scientists are listed below.

FY 2014 Accomplishments

Food Safety, Food Biosecurity, and Methods Development

- Identified Shiga-toxin genes (*stx1* and *stx2*) and intimin genes (*eae*) in O157:H7 and non-O157:H7 Shiga toxin producing *Escherichia coli* (STECs) from humans, foods, and dairy cattle.

- Detected six multi-drug-resistant Salmonella enterica serovars that harbored integrons with dihydrofolate reductase (dhfr) and aminoglycoside adenylyl transferase (aadA2), multiple replicon plasmids and 19 virulence genes from a turkey production facility.

- Identified, for the first time, 603 differentially expressed proteins from *Campylobacter jejuni* following acid shock and investigated them by functional pan-genome analysis.

- Sequenced whole genomes of poorly and robustly colonizing *C. jejuni*; a single nucleotide polymorphism was found in a Na+/H+ antiporter family protein.

- Demonstrated that, like human norovirus, feline and canine norovirus have a winter seasonality and indicated that sunlight (temperature and UV index) may inactivate the virus in the environment and stop norovirus transmission in those animals.

- Showed that *Salmonella* spp. containing a VirB/D4 type 4 secretion system (T4SS) were able to down-regulate antimicrobial response-associated factors in cultured macrophages, likely contributing to their increased ability to survive in macrophages.

- Isolated and characterized *Bacillus* spp. from over-the-counter weight-loss dietary supplements; they have been submitted to CFSAN for genome sequence analysis.

- Characterized *Salmonella* Enteritidis outbreak strains in collaboration with ARL and CDC, using MLVA and Pulsed Field Gel Electrophoresis (PFGE) methods; PFGE alone was unable to distinguish outbreak isolates.

- Evaluated different subtypes of plasmids to identify factors that contribute to the spread of antimicrobial resistance and virulence in *Salmonella enterica*.

- Demonstrated that nontyphoidal *Salmonella* Javiana encodes a cytolethal distending toxin; deletion mutants in the cdt genes were unable to mediate cell cycle arrest. CdtB produced from these strains may play an important role in pathogenesis in host cells.

- Demonstrated that cytolethal distending toxin-positive *Salmonella* serovars cause a higher degree of vacuolization and autophagy in macrophages.

- Conducted molecular studies and whole genome sequencing of multidrug-resistant *Staphylococcus aureus* isolates from Pakistan that were highly resistant to fluoroquinolones and discovered new mutations that contribute to resistance.

Antimicrobials and Pharmaceutical Products

- Studied the impacts of benzalkonium chloride and ciprofloxacin on EmmdR of *Enterobacter cloacae* in an *in vitro* pharmacodynamic model.

- Characterized genotypes, antimicrobial resistance, and virulence genes of methicillin-resistant *S. aureus* strains isolated from imported meat.

- Completed studies on the survival and susceptibility of *Burkholderia cepacia* complex in chlorhexidine gluconate and benzalkonium chloride.

- Examined factors that influence the spread of antimicrobial resistance plasmids from multidrug-resistant *Salmonella* strains to susceptible bacteria, including the influence of antimicrobial exposure and plasmid genetics on the efficiency of plasmid transfer.

- Constructed computerized homology models to study the roles of point mutations on fluoroquinolone resistance in *E. coli* isolates from imported shrimp; only the mutations at codons 83 and 87 in gyrase genes increased fluoroquinolone resistance.

- Determined that variations among mutant strains resulting from resistance selection are found in all aspects of metabolism of *C. perfringens.*

- Demonstrated that changes in metabolic activities of *C. perfringens* were correlated with altered expression of various genes in fluoroquinolone-resistant mutants.

Microbes and Host Interactions

- Developed non-animal models of microbiome interaction and epithelial barriers in the gastrointestinal tract for the risk assessment of FDA-regulated products.

- Developed a multiple analyte assay for culture supernatants and blood sera to detect immunotoxicity.

- Found by scanning and transmission electron microscopy of *Bacillus anthracis* spore-infected primary cells that spore internalization was most rapid in SAEC cells, followed by InMyoFib and NHEK cells.

- Identified, by transcriptomic and microRNA profiling analysis, cell-specific and common biomarker genes in *B. anthracis* spore-infected primary cells; *in vitro* changes in expression levels suggest a possible link between these genes and anthrax lethality.

- Characterized a metallo-β-lactamase as a major contributor to the degradation of the veterinary cephalosporin ceftiofur by a *Bacillus cereus* bovine intestinal isolate.

- Characterized properties of mutants of an FMN-dependent azoreductase from *Enterococcus faecalis*.

- A new concept paper was approved to investigate the use of nanoparticles in enhancing gut immunity to a norovirus vaccine.

- Identified, using real-time polymerase chain reaction (PCR) and high-throughput DNA sequencing, shifts in intestinal microbiota associated with diet and lean or obese state in a rat model for obesity.

Office of Women's Health Projects

- Developed a defined medium that simulates vaginal secretions and supports the growth of *Lactobacillus* species and clinical strains of *S. aureus* from cases of menstrual toxic shock syndrome; it also supports the production of toxic shock syndrome toxin-1 (TSST-1).

- Quantitatively determined TSST-1 production of approximately 30 clinical strains of *S. aureus*, utilizing both ELISA and densitometry methodologies.

- Measured the activation of cell stress responses to nanoparticles made of poly (lactide-*co*-glycolide)-polyethylene glycol (PLGA-PEG) and graphene oxide-polyethylene glycol (GO-PEG) for drug delivery to *Candida albicans* infected vaginal epithelial cells.

Environmental Biotechnology

- Analyzed fluoroquinolone inactivation pathways used by drug-resistant bacteria from wastewater.

- Elucidated a microbial mechanism in response to Deepwater Horizon BP crude oil; identified proteins involved in the degradation of crude oil hydrocarbon components and other cellular physiological responses.

- Elucidated pleiotropic and epistatic function of a ring-hydroxylating oxygenase in the polycyclic aromatic hydrocarbon-metabolic network (PAH-MN) of *Mycobacterium vanbaalenii*.

Nanotechnology

- Evaluated and compared conventional methods for the quantitation of bacterial cells after exposure to metal oxide nanoparticles.

- Analyzed intestinal gene expression of rats subchronically exposed to silver nanoparticles; smaller sizes and lower doses decreased expression of immunomodulatory genes, especially in females.

- Demonstrated that nanoparticles can cross the intestinal barrier (epithelial cells) and modulate the gene expression of permeability related genes.

- With the NanoCore facility, evaluated migration of silver nanoparticles from polarized intestinal epithelial cells and intestinal mucosal tissue by inductively coupled plasma mass spectrometry.

- Demonstrated anti-viral effects of different sizes and dosages of silver nanoparticles against caliciviruses and bacteriophages, used as surrogates for noroviruses and resident gut viruses, respectively.

- A protocol was approved to determine whether the durable nanoparticle bioaccumulation in macrophages increases susceptibility to bacterial infection.

Center for Tobacco Products

- Studied metabolism of smokeless tobacco products by oral bacteria and analyzed nicotine and nicotine metabolites.

- Performed Ames test on smokeless tobacco products before and after metabolism by oral bacteria.

- Developed a method to isolate oral bacterial genomic DNA from oral mucosa swab samples and performed 16S rRNA gene amplification on the samples.

- Found that several bacteria in smokeless tobacco products are nitrate reducers; nitrate and nitrite reduction may contribute to formation of tobacco-specific nitrosamines.

FY 2015 Plans

Food Safety, Food Biosecurity, and Methods Development

- Determine the effect of sunlight on the environmental survival of noroviruses and contamination of farm produce.

- Determine the impact of plasmid-encoded genes on *Salmonella* pathogenicity, using *in vitro* models, and identify factors that are important to the transfer of virulence

and resistance plasmids among enteric pathogens.

- Perform whole-genome sequence analysis of *B. cereus* from dietary supplements.

- Validate multiplex real-time polymerase chain reaction with FDA's Office of Regulatory Affairs (ORA) and Center for Food Safety and Nutrition (CFSAN) for detection of *Salmonella*, *Shigella*, *E. coli* O157:H7, *C. jejuni, Vibrio cholerae, Yersinia enterocolitica, Listeria monocytogenes, S. aureus,* and *B. cereus* in foods.

- Investigate gene diversity in non-O157:H7 Shiga-toxin producing *E. coli*, using an FDA-ECID Affymetrix microarray biochip, and compare the profile with *E. coli* O157:H7.

- Characterize fluoroquinolone resistance in multidrug-resistant *Acinetobacter* spp. isolated from imported shrimp.

- Molecular serotype Shiga-toxin producing *E. coli* from humans, food, and dairy cattle by allelic discrimination of somatic lipopolysaccharide (O-antigen) and flagellar (H-antigen) based on the *fliC*, *wzx* and *wzy* genes.

- Identify "adulterant" virulence genes (Shiga toxins, hemolysins, and intimin) and their alleles that contribute to Shiga-toxin producing *E. coli* pathogenicity.

- Identify the markers associated with methicillin-resistant *Staphylococcus epidermidis* biofilms on medical devices exposed to antibiotics by a multi-omics approach.

- Perform whole-genome sequence analysis of more multidrug-resistant *S. aureus* isolates from Pakistan and compare their genomes for differences in virulence genes, antimicrobial resistance genes, and pathogenicity island profiles.

- Determine the antibacterial effect of migrated silver nanoparticles/silver ions from food-contact materials in consumer-use products, assessing the potential exposure risk and impact on gut commensal microbiota.

Antimicrobials and Pharmaceutical Products

- Sequence plasmids harboring the New Delhi Metallo gene ($bla_{\text{NDM-1}}$) in *E. coli* isolates from companion animals.

- Continue to explore proteomic profiles of *Burkholderia cenocepacia* AU1054 strains in benzalkonium chloride to understand the resistance mechanism; determine the degradation of benzalkonium chloride by the *Burkholderia cepacia* complex.

- Determine the impact of low levels of tetracycline on a bioreactor of the human intestinal microbiota, using "omics" and analytical chemistry methods.

- Characterize cephalosporin-resistant *Salmonella enterica* from imported food samples and investigate the transferability of antibiotic-resistance genes to other enteric bacteria.

- Identify mechanisms for antimicrobial resistance and the role of efflux pumps in *L. monocytogenes* isolates from foods.

- Evaluate antimicrobial susceptibilities of *C. perfringens* strains from different sources.

Microbes and Host Interactions

- Investigate the use of nanoparticles to enhance immunity to a norovirus VLP vaccine.

- Initiate a study on the role of influenza virus on secondary bacterial pneumonia.

- Extend studies on the bioavailability of antimicrobials and the impact of antimicrobial residues on the human intestinal microbiota.

- Evaluate the potential antimicrobial resistance selection in human intestinal microbiota following long-term exposure to residual concentrations of tetracycline, as a part of human-food safety assessment.

- Identify microRNAs involved in the control of gene expression and anthrax disease development processes of the three forms of anthrax and identify the biomarkers that could possibly be used as targets for therapeutic interventions.

- Develop a method for detection of intestinal virus and phages by real-time PCR.

- Study the influence of commensal Bacteroidetes and Firmicutes on pro-inflammatory responses of intestinal epithelial cells and dendritic cells to *Clostridium difficile* infection, to better understand the efficacy mechanisms of fecal microbiota transplantation.

- Evaluate the role of shifting intestinal microbiota populations on the metabolism of the soy isoflavone daidzein to equol and its correlation with mammary tumor formation.

Office of Women's (OWH) Health Projects

- Initiate a recently funded grant from OWH (collaboration with Center for Devices and Radiological Health (CDRH) by hiring a post-doctoral fellow and begin a comprehensive analysis of tampon involvement in *S. aureus* growth and TSST-1 toxin production using the CDRH-recommended procedures.

- Evaluate differences in cytotoxic mechanisms in vaginal epithelial cells induced by PEGylated graphene oxide nanoparticles and pristine graphene nanoparticles as potential intravaginal drug-delivery vehicles.

- Characterize the efflux pump activities in uropathogenic bacteria and investigate the genetic mechanism of efflux pump regulation in these organisms.

Environmental Biotechnology

- Continue analysis of the mechanism of fluoroquinolone degradation by drug-resistant bacteria from wastewater.

- Continue analysis of the mechanism behind the bacterial degradation of BP crude oil by analyzing genomic, proteomic, metabolic, and bioinformatics data.

Nanotechnology

- Conduct *in vitro* assays to screen the human skin microbiota cell toxicity in the presence of nanoscale materials and determine the effect of nanomaterials in cosmetics on human skin microbial ecology.

- Investigate antimicrobial properties of zinc and titanium dioxide nanoparticles that may work in synergy with antibiotics in multidrug-resistant *Enterococcus* and *Staphylococcus* spp. and investigate transcriptomic gene expression in human cell lines after exposure.

- Collaborate with the NanoCore facility to detect changes in the ultrastructure of human ileal tissue explants due to nanoparticles by electron microscopy.

- Continue collaboration with the Arkansas Research Consortium in Nanotechnology to test graphene-induced toxicity to the intestinal microbiota and the gut-associated immune response, using an *in vitro* model system.

- Determine the effect of subchronic oral exposure to silver nanoparticles on the intestinal microbiota by next generation sequencing.

- Characterize structure-function relationships of drug nanocrystals for surface potential, crystallinity, and aggregation state at various physiological pH values.

- Evaluate drug nanocrystal effects on epithelial cell permeability, cell proliferation, and mucoadherence using *in vitro* and *ex vivo* culture models.

- Determine the effect of drug nanocrystals on intestinal commensal microbiota.

- Develop a method to evaluate bactericidal activity of food-contact materials containing silver nanoparticles and silver ions.

- Conduct silver nanoparticle exposure studies on gut microbiota, using a continuous fermentation system, to assess the metagenomic profiles of the gut viral component.

- Determine changes in the capsids of viruses during nanomaterial exposure.

- Study the pro-inflammatory responses of vaginal tissues to PEGylated drug-delivery nanoparticles in mice to assess safety of these products during active yeast infections.

- Determine whether preexposure of mice to silver, gold, and silica nanoparticles will make them more susceptible to bacterial infections.

Center for Tobacco Products

- Continue to study the effects of oral bacteria on the toxicity of smokeless tobacco products and analyze metabolites of the smokeless tobacco products.

- Determine the effects of smokeless tobacco products and tobacco-specific nitrosamines on oral bacterial ecology of animals by using molecular biological methods.

Contribution to FDA's Strategic Priorities/Goals

Research in the Division of Microbiology contributes to FDA Strategic Goals 1, 2, 3 and 4.
FDA Strategic Goal 1 (*Enhance Oversight of FDA-Regulated Products*)

- Characterization of plasmids from *E. coli* that harbor the New Delhi metallo-beta-lactamase (NDM) gene has important public health significance because these plasmids may play a significant role in carbapenem resistance. Carbapenem-resistant Enterobacteriaceae (CRE) have been highlighted in the CDC's 2013

Antimicrobial Threat Report as an “Urgent Threat” to public health.

- Molecular typing methodologies, involving next generation genotyping combined with high efficiency target-capturing methods, will be useful in high-throughput characterization of foodborne pathogens.

- Better methods to confirm norovirus survival in animals and foods will predict viral recombination, improve food safety, and assist FDA in resolving legal impasses.

- Recovery and detection methods for the *Burkholderia cepacia* complex found in pharmaceutical environments will be used by the FDA and industry.

- Research on the impact of low levels of tetracycline on human fecal materials will assist the FDA`s strategies for antibiotic use and drug residue limits for food animal products.

- New *in vitro* virulence assays will screen isolated pathogens from FDA-regulated products for their pathogenicity and an understanding of the molecular basis of altered pathogenicity in antimicrobial-resistant strains of bacteria will improve drug safety.

- The ability to understand how foodborne pathogens evolve increased virulence and resistance to antimicrobials is important to achieve FDA’s goal of implementing a new prevention-focused food safety system to protect public health.

- The dynamics of pre- and postharvest *Salmonella* colonization in poultry can be useful in establishing strategic critical control points to limit the prevalence of this pathogen, particularly in the absence of sub-therapeutic antibiotics.

- Newly developed *in vitro* and *ex vivo* models will provide a clearer understanding of how drug residues, probiotics, dietary supplements and xenobiotics affect the intestinal microflora and immune responses.

- Establishing science-based minimum standards for conducting hazard analysis of products containing silver nanoparticles is a step towards FDA readiness to evaluate innovative technologies for product assessment.

FDA Strategic Goal 2 (*Improve and Safeguard Access to FDA-Regulated Products to Benefit Health*)

- Migration of nanomaterials from consumer-use products may lead to exposure of mucosal surfaces and impact intestinal health, including effects on the commensal

microbiota and host immune function; thus studies of nanotechnology will need to consider these effects.

- Evaluating the efficacy of combination regimens of antibiotics and chemicals by evaluating bacterial killing and the suppression of multidrug-resistant bacteria in an *in vitro* pharmacodynamics model is important for understanding the mechanism of resistance.

- Investigating the effects of fluoroquinolones on pathogenic bacteria, particularly mutations associated with antibiotic resistance, will be valuable for understanding the emergence of more virulent strains of bacteria.

- Metabolism of veterinary antimicrobials by intestinal microbiota and wastewater bacteria may affect development of resistance. Since the FDA sets drug residue limits for animal products, this will assist in writing strategies for antibiotic use.

- Microbial shifts during interaction of silver nanoparticles with the gastrointestinal tract will lead to identification, quantitation, and molecular characterization of gut mucosal exposure to nanomaterials in foods and other FDA-regulated products.

- Emerging nanotechnology for intravaginal drug delivery, which promises to improve treatment of diseases in women, is important to understand immune responses to intravaginal nanomaterials during yeast infections coinciding with use of the products.

- Understanding modulation of pro-inflammatory responses in epithelial and dendritic cells by commensal microbes during *C. difficile* infections will allow better regulation or replacement of fecal microbiota transplants for pseudomembranous colitis.

- Examining the effect of nanoparticle pre-exposure in mice followed by bacterial infection will help in assessing whether nanoparticles interfere with normal pathogen clearance from the system or increase susceptibility to bacterial infections.

- Examining the interference of nanoparticles with bacterial quantitation will help assess bacterial contamination in foods, drugs, and cosmetics containing nanoparticles.

- Rapid and sensitive methods to detect enterotoxin-producing *B. cereus* in dietary supplements will assist in developing guidelines for their regulation.

- Investigating the effect of the chemical structure of a drug on development and alteration of virulence will lead to improving the treatment of bacterial infections and prevent development of more resistant virulent strains.

- Comparative differential proteome analyses will provide useful information for the molecular mechanism of *C. jejuni* survival in the chicken intestine and help prevent *C. jejuni* colonization of chickens.

- Determination of the contribution of bacterial plasmids to increased virulence and the factors that affect their spread could lead to new strategies to identify and control pathogens with increased virulence.

- Determining how shifts in the intestinal microbiota associated with obesity affect isoflavone metabolism will improve our understanding of the combined effect of high soy diets and obesity on breast cancer formation in Western populations.

FDA Strategic Goal 3 (*Promote Better Informed Decisions About the Use of FDA-Regulated Products*)

- Rapid detection of Shiga toxins in foods will make it possible to prevent outbreaks of O157:H7 and non-O157:H7 Shiga-toxin producing *E. coli* and provide data for an FDA risk-assessment model for foodborne pathogens.

- Norovirus research will help redirect FDA inspection efforts and improve food safety.

- Next-generation sequencing of the microbial population in the intestine during silver nanoparticle exposure will reveal changes in the population of commensal bacteria, have a regulatory impact on warning labels and reduce intestinal side effects.

- Research on nanoparticle exposure to bacterial susceptibility will help in developing ICH-S8, ASTM, ISO standards and regulatory guidance policy for the pharmaceutical industry.

- The whole-genome sequence analysis of methicillin-resistant *S. aureus* isolates, along with the detection of toxin and virulence gene markers, will be helpful in developing strategies to control *S. aureus* infection.

- FDA will gain a clearer understanding of how drug residues, probiotics, dietary supplements, and xenobiotics affect the intestinal microflora and human health.

- Genetic analysis of bacteria will assist in determining the route of food contamination; understanding antimicrobial resistance and virulence will help to develop intervention strategies to improve the safety of foods.

- Environmental biotechnology research will help document environmental fate and toxicity before FDA approval of new drugs and other medical products, as indicated by

FDA's Center for Drug Evaluationa and Research (CDER) and Center for Veterinary Medicine (CVM) "Guidance for Industry" documents.

- Determination of the effect of nanomaterials in cosmetics on human skin microbial ecology will enhance our scientific knowledge of the safety and toxicity of the nanomaterials in cosmetics and provide data to be considered for safety assessment**.**

- By determining if smokeless tobacco product contaminants influence the oral microbiota and epithelium, bacterial biomarkers in oral microflora will be used to evaluate toxic effects of smokeless tobacco products.

- Comparison of gene expression profiles of human cutaneous, gastrointestinal, and pulmonary cell lines after infection with *B. anthracis* will help find biomarkers to enhance product safety and develop counterstrategies to reduce or eliminate mortality.

- Determination of host susceptibility to bacterial infections after exposure to nanoparticles will be helpful in determining whether NP-based investigative new drugs will be safe for human use or increase susceptibility to bacterial infections.

- Determining the feasibility of generating a microbiological standard of testing for feminine tampons should promote an increased level of assurance to the consumer as well as the manufacturer and CDRH reviewers.

- Division scientists are actively participating in the Arkansas Research Consortium in Nanotoxicity (ARCN); this collaboration will help FDA to provide leadership on safety and regulatory status of food and drug ingredients and food-contact substances containing graphene.

Division of Neurotoxicology Summary of Activities

Merle G. Paule, Ph.D., Director
870-543-7203
Merle.Paule@fda.hhs.gov

Introduction

The National Institute of Mental Health estimates that one in four American adults suffer from a diagnosable mental disorder in any given year and that one in three will experience some form of mental disorder during their lifetime. Fifty-million Americans have a permanent neurological disability that limits their daily activities. The number of persons suffering from Alzheimer's, Parkinson's, and other neurodegenerative diseases is increasing dramatically as our population ages. Disability from depression alone exceeds that of diabetes, hypertension, gastrointestinal, and lung diseases combined. Conservative estimates put the financial cost of brain-related dysfunction in the U.S. at well over half a trillion dollars per year. Thus, diseases and disorders of the brain represent enormous societal burdens, both economically and in terms of human suffering. The known and suspected causes of brain-related disorders include exposures to chemicals—including therapeutic drugs and drugs of abuse—food additives, food products, cosmetic ingredients, pesticides, and naturally occurring substances. Each year millions of children are exposed to anesthetics and sedatives that have been shown in pediatric-animal models to cause significant nerve-cell death and subsequent brain dysfunction. Nanomaterials are entering our world at an ever-increasing pace, yet little is known about their potential toxicity. Addictive behaviors associated with tobacco products continue to take their toll.

The number of FDA-regulated chemicals that can affect the nervous system runs well into the thousands and chemicals that are known or suspected causes of brain-related disorders are vital to the national economy and our quality-of-life. Our challenge, thus, is to determine at what levels of exposure and under what conditions these compounds can be used effectively while maximizing safety.

The ultimate goals of the Division of Neurotoxicology are to understand the biological pathways relevant to the expression of neurotoxicity in order to identify relevant, yet practical, biomarkers. Developing methods to help identify potential toxicities is critical for the assessment of neurotoxic risk, the development of informed safety guidelines, and the development of protective and therapeutic strategies.

The strategies employed for achieving these goals often involve multidisciplinary approaches that capitalize on the expertise of Division personnel which includes: neurochemistry, molecular neurobiology, neuropathology, neuro and behavioral pharmacology, neurophysiology, experimental psychology, and multi-modal bio-imaging. Fortunately, technological advances are continuing to provide new tools with which to better study and understand the causes and pathologies associated with brain-related diseases and to better define the biological pathways involved. Efforts to develop sensitive, high-throughput systems for screening potential neurotoxicants have been developed (brain cell cultures, zebrafish, rodent neural stem cells) or are well underway (human and nonhuman primate-neural stem cells). Methods are being employed to assess the addiction potential of tobacco product constituents. Use of these advances will provide the tools to assess the risks associated with the use of regulated products and inform actions to protect and improve public health.

FY 2014 Accomplishments

In partnership with FDA's Center for Drug Evaluation and Research (CDER) colleagues, Division staff continued studies on the neurotoxicity associated with pediatric anesthetics utilizing both *in vitro* (rodent and human neuronal-cell cultures) and *in vivo* (rat and nonhuman primate) approaches by expanding studies to include the commonly used anesthetic agents sevoflurane and propofol. The data obtained continue to be important for the regulatory needs of the agency and importantly, our approach is beginning to identify strategies that may prevent or ameliorate anesthetic-induced neurotoxicity. This year we extended our studies utilizing the imaging compound, FEPPA, that allows us to visualize aspects of brain inflammation in a non-invasive fashion using Positron Emission Technology (PET) imaging technology. We are, thus, now able to follow the time course—in both rodents and primates—of neuroinflammation associated with pediatric exposures to general anesthetics and to begin to explore ways to ameliorate such effects. The utilization of PET imaging brings us closer to our goal of being able to translate preclinical findings to the clinical setting. Coupling Computerized Tomography (CT) technology with PET technology brings enhanced mapping detail to the process and we added new instrumentation during the past year to expand our efforts in that direction.

In studies exploring the biological mechanisms associated with pediatric anesthetic-induced neurotoxicity we have continued to demonstrate in virtually all of our models that the anti-oxidant and mitochondrial-stabilizing agent, acetyl-L-carnitine, exerts significant neuroprotective properties when given prior to and during pediatric anesthesia. Studies are now underway to assess the effects of developmental exposures to acetyl-L-carnitine on brain function in our nonhuman primate model. In these studies we are employing NCTR's battery of cognitive functions tests (the Operant Test Battery; OTB) that are also being used in collaborating clinics studying children who have experienced general anesthesia at a young age.

The use of animal neural stem cells has figured prominently in much of our work on pediatric general anesthetics and other agents with a view towards further elucidating the cellular and subcellular effects underlying exposure-associated neurotoxicity. This year we have incorporated human neural stem cells into these studies and are now providing information not only about how anesthetics might impact nerve-cell growth, differentiation, and proliferation but also about species comparability and the ability to extrapolate animal findings to human systems.

Employing Magnetic Resonance Imaging (MRI) with a very powerful magnet (7 Tesla), prototypic neurotoxicants were used in studies to induce classic neuropathology and the MRI was used to obtain preliminary information in living animals about the location, onset, severity, and time-course of notable changes in MRI signals. Here, initial studies comparing brain images obtained using MRI with actual brain-tissue slices using traditional neuropathology techniques have been completed. The data demonstrate that the use of MRI in such a fashion will provide additional power for helping to detect neurotoxicity. By identifying areas of abnormal MRI signals in the brain, subsequent neuropathological assessments using tissue slices and traditional staining techniques can be targeted towards those areas, thereby maximizing chances of detection while minimizing effort. In addition, each animal can serve as its own control and be imaged repeatedly in a non-invasive manner, thus, reducing the number of animals needed and providing comprehensive life-cycle information on brain tissue responses to chemicals. An initial description of this work has already been provided to the regulatory science community.

Significant progress was made in the development of novel histochemical tracers to aid in the evaluation of brain pathologies: a novel fluorescent tracer (Fluoro-Turquoise conjugated gelatin) was developed for visualizing brain vasculature and endothelial cells. This compound was used in a rat model to characterize the effects of a prototypical excitatory neurotoxicant, kainic acid. Previously, this approach was used to localize cells surrounding and supporting blood vessels in the brain and, thus, provided opportunity for studying the reaction of these important cells to other neurotoxicant exposures and to study the effects of a variety of drug classes on the progression of amyloid plaque deposition in a transgenic mouse model of Alzheimer's disease. Such efforts allow for the assessment of the role of amyloid plaque aggregation and deposition in the expression of neurotoxicity.

The use of the dye Fluoro-Jade C (for labeling dead and dying nerve cells) was previously only known to be effective in fixed tissue. We recently showed that it also works in fresh (unfixed) tissue as well as in living cells in culture. These observations are important since they suggest that it may now be possible to assess the health of living cells in a rapid and high-throughput fashion. Changes in gene and protein expression in sick cells identified in this manner can then be assessed: this is not possible in fixed tissue.

At exposures matching or near human therapeutic levels, we are seeing few adverse effects of chronic methylphenidate (MPH) treatment given throughout adolescence on cognitive function (OTB performance) in a nonhuman primate model. PET imaging studies, designed to examine the integrity of important neurotransmitter systems, have begun and will continue while these animals continue MPH administration. In light of the current widespread use of MPH to treat Attention Deficit and Hyperactivity Disorder (ADHD), such studies are critical in providing important information about the safety of its long-term administration. Cognitive functions in children are also being assessed using the NCTR OTB—the same instrument used on our nonhuman primates at NCTR. These studies are being carried out at our laboratory at nearby Arkansas Children's Hospital and in laboratories at the Mayo Clinic and the University of Iowa where the effects of pediatric general anesthesia are being studied. Such studies are exemplary of translational neuroscience and highlight the cross-species, cognitive function comparison capabilities within the Division.

Two behavioral pharmacology laboratories to support the needs of the Center for Tobacco Products neared completion. A rodent behavioral pharmacology laboratory will serve to provide information on the ability of tobacco product constituents to release neurochemicals thought to contribute to their rewarding effects. A related nonhuman primate laboratory will assess the ability of tobacco product constituents to actually produce and maintain addictive behaviors.

In partnership with the Health and Environmental Sciences Institute of the International Life Sciences Institute, plans have been developed to initiate—using the prototypic neurotoxicant trimethyltin—a protocol to attempt to identify biomarkers of neurotoxicity having potential clinical utility. The focus will, thus, be on those biological signals that are available using minimally-invasive sampling techniques such as those involving the collection of bodily fluids and imaging techniques. This effort involves partners from government, industry and academia and has resulted from extensive collaborative consultation.

FY 2015 Plans

Division efforts in FY 2015 will focus on studies addressing the following:

- The effects of developmental exposures to pediatric general anesthetics on subsequent complex brain function in rodent and nonhuman primate models.

- The effects of adult exposures to general anesthetics on subsequent complex brain function in rodent models.

- Expanding the utilization of *in vitro* models including animal and human neural stem cells, blood-brain barrier models, and the zebrafish developmental-neurotoxicity model to study prototypic neurotoxicants including general anesthetics.

- The efficacy and toxicity of a variety of potential anti-Alzheimer's agents using transgenic-mouse and rat models.

- The development and use of novel histochemical tracers to monitor the health of important nervous-system structures in both fresh and fixed tissue.

- Utilization of state-of-the-art imaging capabilities to provide new insights into the events contributing to neurotoxicity and neuroprotection.

- Identification of MRI approaches to support regulatory science efforts by developing standards for using MRI/Magnetic Resonance Spectroscopy (MRS) signals to direct traditional neuropathological assessments.

- The ability of nanomaterials and other regulated products to affect the integrity of a variety of nervous system related models including the blood-brain barrier *in vitro* and *in vivo*.

- The relationship between performance of the tasks that comprise the NCTR Operant Test Battery (OTB) and clinically-relevant psychological tests to further assess the translatability of the OTB.

- Rodent and nonhuman primate behavioral pharmacology laboratories to support Center for Tobacco Product studies on the ability of tobacco product constituents to engender and/or maintain addictive behaviors.

Contributions to NCTR's and FDA's Strategic Priorities/Goals

The bulk of the research conducted by the Division of Neurotoxicology directly supports NCTR's Strategic Goal of *Advancing Scientific Approaches and Tools Required to Support Public Health*, a goal which directly supports FDA's Core Mission and Goals.

The development of sophisticated imaging approaches, alternative preclinical models (zebrafish, neural stem cells, and *in vitro* blood-brain barrier models), cross-species metrics of brain function, and the integration of omics approaches to identify novel markers of neurotoxicity all support FDA's Goal and Objective: Goal 1 (*Enhance Oversight of FDA Regulated Products; Objective 1.1 Increase the use of regulatory science to inform standards development, analysis and decision-making*). Developing and refining animal models and providing techniques for monitoring and detecting

adverse effects associated with the use of regulated products are clear objectives under this Agency goal.

The Division's work on the toxicity of general anesthetics and sedatives used in a pediatric context and its studies on the effects of developmental exposures to methylphenidate and other agents also supports the FDA Goal and Objective Goal 2: (*Improve and Safeguard Access to FDA-Regulated Products to Benefit Health; Objective 2.2 Improve the Effectiveness of the Product Development Process*) and the priority to "Expand Efforts to Meet the Needs of Special Populations" by conducting research directly relevant to children. Our training of students, visiting scientists, and postdoctoral fellows contributes to all of these goals.

Division of Systems Biology Summary of Activities

William B. Mattes, Ph.D., D.A.B.T., Director
870-543-7681
William.Mattes@fda.hhs.gov

Introduction

The Division of Systems Biology focuses on the development and evaluation of new technologies and the identification of new biomarkers to support the FDA mission. The Division is divided into three branches:

1. Biomarkers and Alternative Models Branch
2. Innovative Safety and Technologies Branch
3. Personalized Medicine Branch

The Division is comprised of a multidisciplinary group of toxicologists, analytical chemists, physical chemists, computational modelers, etc. The goals of the Division are to use systems biology approaches and emerging science and technology to:

- Find new translational biomarkers to a) improve detection of unsafe drugs and other FDA-regulated products and b) improve the identification of disease onset and its progression to enable better medical intervention.

- Develop and evaluate innovative methods to detect unsafe products, advance the identification of infectious disease, and enhance diagnostic procedures.

- Determine the impact of differences in the responses of species and human sub-populations on current assessments of drug safety and efficacy.

- Evaluate the potential of agents of interest to the FDA to induce developmental toxicity. This is accomplished by the use of well-established methods and development of new approaches including use of stem cells.

FY 2014 Accomplishments

Biomarkers of Tissue Injury:

1. Liver

Liver injury caused by drugs remains not only a major reason for failure during the drug-

development process (and risk for clinical-trial subjects) but also a patient-management issue with approved medications. Existing tests for liver damage are sensitive, but not specific, and do not provide information regarding the severity of the damage or the likelihood of repair. Thus, new biomarkers are needed.

- Examining animal models we found a dramatic increase in HMOX1 levels in plasma from rats overdosed with acetaminophen (APAP) that was correlated with liver damage. This study provided insights into the mechanisms of APAP-induced liver toxicity and identified a unique protein, HMOX1, as a potential plasma biomarker of liver injury.

- Addressing the question of dietary effects on drug toxicity we found that epigallocatechin gallate (EGCG), the major component of green tea, only inhibited function in rat mitochondria when they were already compromised (i.e.. swelling) . This suggests that EGCG might potentiate the hepatotoxicity of drugs that are causing minimal liver damage.

- An important goal is to develop preclinical biomarkers distinguishing three types of compounds [i.e., those that cause overt toxicity in multiple species, those that induce hepatotoxicity only in humans (idiosyncratic) and those that are known to have no adverse effect on the liver]. Forty-one metabolites, identified in previous hepatotoxicity studies, were semi-quantified and used to build models to predict hepatotoxicity using the data from the liver toxicants and non-liver toxicants. This biomarker model showed promise in diagnosing acute and idiosyncratic hepatotoxicity.

- To investigate the translational nature of such biomarkers, we have undertaken two studies exploring microRNA (miRNA) responses in humans under conditions of hepatoxicity. In collaboration with Dr. William Lee and the Drug-Induced Liver Injury Network (DILIN) we have examined urine miRNAs in patients studied by the Acute Liver Failure Study Group. While the results are preliminary, it seems that miRNA patterns can discriminate between survivors and non-survivors. In another clinical study, serum, and urine samples were obtained from 1) healthy pediatric patients, 2) those exposed to therapeutic doses of acetaminophen (APAP), and 3) those that had overdosed with APAP. Significant increases in the serum and/or urinary level of several species of miRNA were observed upon APAP overdose. While in many cases the temporal response of such miRNAs were similar to that of other biomarkers (e.g. APAP-protein adducts), some miRNAs had a response suggestive of an early biomarker. Our results suggest that miRNAs might provide needed information to those developing drugs and to clinicians managing patients undergoing drug-induced liver injury.

- In a metabolomics analysis of such pediatric patients conducted in parallel, we observed significant increases in serum ALT, APAP protein adducts, and acylcarnitines (important in energy metabolism) in those overdosed children that received delayed antidote treatment (NAC, N-acetylcysteine). The perturbations in long chain acylcarnitines suggest that mitochondrial injury and associated impairment in the *β*-oxidation of fatty acids are clinically relevant biomarkers of APAP-induced mitochondrial toxicity.

2. Heart

Doxorubicin is a potent anticancer drug that can cause chronic heart damage in humans. There is a need to identify early indications of heart damage so that treatment can be modified or discontinued.

- A mouse model of drug-induced cardiac injury was developed. Changes in the expression levels of genes and miRNAs (genomics) and proteins (proteomics) in the heart were associated with drug-induced cardiac injury and identified as candidate biomarkers of early cardiac injury and may lead to a new understanding of the mechanism behind doxorubicin toxicity. Metabolomics analysis of the plasma and tissue revealed potential biomarkers in both samples that preceded overt heart damage. In particular, 3 of 7 short acylcarnitines including carnitine were increased in plasma but decreased in the heart early in doxorubicin treatment.

Innovative Technology:

1. Infectious agents and antibiotics

Rapid identification of infectious agents in FDA-regulated products continues to be an important public health need.

- RAPID-B™ is a technology that was developed by researchers within this division and has been licensed to a commercial entity. This is a flow-cytometric-based approach using a field-tested machine. A successful internal FDA level-3 validation of the *E. coli* O157 (a variant of a bacterium that causes severe food poisoning) was performed in collaboration with FDA's Office of Regulatory Affairs' Arkansas Regional Laboratory.

- RAPID-B™ was successfully adapted to identification of prions in human blood samples using nanospheres.

- Initial studies have shown success in detecting *Listeria* monocytogenes and *Listeria* spp. using genetic probes.

2. Stem cells

In the past, *in vitro* cell culture testing was limited to immortalized cell lines, or primary cells isolated from tissues. In the case of cell lines, there was always the question of relevance of cellular processes to those of normal tissues, and in the case of primary cells there is always the question of de-differentiation in culture. Stem cell cultures offer the possibility of utilizing well-characterized differentiated cells and/or monitoring differentiation in culture. Our work has explored the utility of both for new assays of chemical safety.

- The mouse Embryonic Stem Cell Test (mEST) has been used to examine agents that induce developmental toxicity. The current assay uses differentiation to cardiomyocytes as an endpoint; we have examined the use of differentiation to osteoblasts as a parallel endpoint. Our results suggest that differentiation to osteoblasts may provide confirmatory information in predicting embryotoxicity.

- In another study, human induced pluripotent stem (iPS) cell-derived cardiomyocytes have been evaluated for their ability to evaluate the potential of regulated products for cardiotoxicity. The toxicity of mainstream cigarette smoke condensates (CSCs) was assessed in iPS derived cardiomyocytes with cellular function assays and cardiomyocyte-specific endpoints. The CSC treatments reduced cell viability and resulted in dose-dependent changes in the beat rate as assessed by a real-time cellular impedance measurement. Global gene expression analysis of cardiomyocytes treated with CSCs using Next Generation Sequencing identified dysregulation of genes for multiple cardiac ion channels, including major genes from potassium and calcium channels.

3. Computational modeling

To improve early screening of molecules for unwanted toxicity, new *in silico* modeling approaches are being developed.

- We have continued to improve upon our patented modeling approach (3D-QSDAR) to models that are more accurate and useful than previously published models. We have published the results modeling binding to the progestin, androgen, estrogen, and aryl-hydrocarbon receptors. Furthermore, we have extended this approach to 3D-SDAR models of phospholipidosis, and binding to the hERG channel protein; both models were built and statistically validated with external data. Note that drug-induced phospholipidosis, observed preclinically and clinically, is a concern for the FDA, and compound binding to the hERG channel protein has been shown to have a role in cardiac dysfunction. Thus both models have the potential to augment *in silico* screening and/or regulatory appraisal of NMEs (New Molecular Entities).

- The 3D-QSDAR models offer a unique advantage in being able to predict a chemical toxicophore, and a fascinating finding is that the toxicophores predicted by the hERG channel and phospholipidosis models have true similarity. The possibility that channel binding of some nature may play a mechanistic role in phospholipidosis is being explored.

Species and Sub-population Comparisons and Personalized Medicine:

Since New Molecular Entities (NMEs) are evaluated for safety and efficacy on the basis of both non-clinical and clinical data, it is essential to evaluate how responses in animal models recapitulate those in various human populations. Conversely, to enable personalized medicine, the uniqueness of sub-populations and individuals and how this affects responses (both in terms of efficacy and toxicity) must be better understood. In addition to the rat and mouse studies reported above that evaluated individual differences in response to drugs that induce liver damage, other studies were performed.

1. Species-Comparative Responses

An important question for both research and regulatory practice is which drugs show concordance in human and animal adverse responses and which do not. An approach to data mining FDA submission documents was developed that allows direct comparisons between clinical and preclinical reports of toxicity. Classes of drugs that show concordance in animal and human responses include mTOR inhibitors, while those that do not show concordance include nucleoside reverse transcriptase inhibitors (NRTIs).

2. Sex-Comparative Responses

Sex-related differences have been indicated in the development of cardiotoxicity induced by doxorubicin. However, mechanism(s) underlying differences in susceptibility to doxorubicin between the sexes is still unclear. A mouse model of sex-related differences in doxorubicin was developed that showed a greater sensitivity to doxorubicin in males than females.

3. Age-Comparative Responses

We previously conducted a study of the mRNA, miRNA, and DNA methylation levels of selected organs of untreated rats, assessed at various time points during their life cycle and in both sexes. Analysis of the miRNAs in kidney showed that not only was there a significant difference in the expression patterns at different ages, but also significant inter-individual differences at the same age.

4. Sub-Population Comparative Responses

Carbamazepine is a drug commonly used to treat epilepsy, among other conditions, but genetic susceptibilities to serious adverse drug reactions (ADRs) have been noted and

there is a need to identify these causative genetic variants. To identify genetic variants that contribute to the risk of carbamazepine-induced ADRs, DNA samples from 30 case subjects and 20 controls were analyzed by using next-generation sequencing technology and validated in a Caucasian cohort. Preliminary results revealed that two clusters of single nucleotide polymorphisms (SNP)s, locating at HLA genes (chromosome 6) and the ESYT2 gene (chromosome 7), are highly associated with carbamazepine- induced ADRs.

Developmental Toxicity:

2-hydroxy-4-methoxybenzophenone (HMB; oxybenzone) is an ultraviolet (UV)-absorbing compound used in many cosmetic products as a UV-protecting agent and in plastics for preventing UV-induced photodecomposition. HMB has been detected in over 97% of randomly collected human urine samples and in the urine from premature infants, and it may have estrogenic potential. Rats were exposed to various doses of HMB in feed throughout pregnancy and lactation, and while some parameters were affected at the highest dose, at possible human exposure levels, HMB does not appear to be a significant reproductive toxicant.

FY 2015 Plans

- It is important to identify and qualify new biomarkers of tissue damage both in species used in preclinical testing and in humans. To this end, new attempts on creating collaborations to access useful human samples will be made. The lack of standards in some technical areas of biomarker discovery stymie the full acceptance of these sciences, so efforts will be made within consortium and other approaches to develop consensus approaches.

- Equally important is the development of new approaches to improve safety evaluation of FDA-regulated products. The use of stem cells will be further explored to examine the utility of cells derived from males or females and those derived from relevant subpopulations.

- We plan to expand the application of our *in silico* modeling approaches to areas that may provide screening opportunities, such as drug-related suicidality and drug-abuse potential.

- Using the newly established mouse model of sex-related differences in cardiotoxicity induced by doxorubicin, we plan to investigate differences in pharmacodynamics and various molecular aspects that might be responsible for heart damage. This information will aid in identifying potential mechanisms responsible for a clinically important question.

- We plan to design and fabricate a mitochondria-specific gene array for the nonhuman primate and the human to characterize species-specific transcriptional

profiles associated with mitochondrial function to predict risk factors of drug toxicity or disease onset in different mammalian species.

- We plan to further explore the study of the mRNA, miRNA, and DNA methylation levels of selected organs of untreated rats, assessed at various time points during their life cycle and in both sexes for transcriptomics-based predictions of sex- and age-related susceptibilities to treatment-induced adverse effects.

- Previous studies suggested that genetic variants associated with carbamazepine-induced adverse reactions are variable in different populations. We plan to perform whole-genome sequencing in 60 patients with carbamazepine-induced adverse reactions and 60 controls from a Chinese population, to identify biomarkers responsible for individual differences in susceptibility to carbamazepine-induced adverse drug reactions in Han Chinese.

- We plan to carry out a comparison of MALDI-TOF imaging of metabolites (both xenobiotic and endogenous) and proteins in tissue slices with histopathology data.

Contributions to FDA's Strategic Priorities/Goals

The work performed in the Division of Systems Biology contributes to FDA's Strategic Goals. The work being done on identifying biomarkers of drug-induced organ injury, developing new stem cell assays, and computational modeling all support FDA Objective 2.1 (*Increase Regulatory Science Capacity To Effectively Evaluate Products*) as well as Objective 2.2 (*Improve the Effectiveness of the Product Development Process*). These efforts and those focused on the improvement of bacteria detection and prion contamination of FDA-regulated products also support Objective 1.1 (*Increase the Use of Regulatory Science To Inform Standards Development, Analysis, and Decision-Making*). Finally the efforts being made in understanding of personalized medicine, and the role of genetics, sex, and age on drug-induced tissue damage support Objective 2.2 (*Improve the Effectiveness of the Product Development Process*).

NCTR Objective 1.1 – Integrated Product Assessment

PI: Ali, Syed F., Ph.D.

Neurotoxicity Assessment of Harmanes, Nonharmane and Nicotine Constituents of Tobacco Smoke in Rats (E0745201)

Responsible Division: Neurotoxicology
Collaborating Divisions/Office: Biochemical Toxicology, Bioinformatics and Biostatistics, Office of Scientific Coordination
Objective(s):

1) Determine if harmane, norharmane, and/or nicotine produce oxidative stress and neurotoxicity in the rat using microdialysis techniques.
2) Determine whether acute or chronic exposure to harmane, norharmane, and/or nicotine produces significant changes in monoaminergic systems in different regions of the rat brain using microdialysis.
3) Determine if exposure to harmane, norharmane, and/or nicotine produces selective neurotoxicity as determined using:
    a. neurochemical biomarkers, such as monoamine levels
    b. cellular biomarkers for oxidative stress such as free radicals, reactive oxygen species, and reactive nitrogen species
    c. molecular biomarkers, such as changes in genomic and proteomic expression as detected using microarrays.
4) Conduct neurobehavioral assessments during early and late adolescence, adulthood and after nicotine challenges and correlate these changes with *in vivo* microdialysis findings.

PI: Ali, Syed F., Ph.D.

Neurotoxicity Assessment of Graphene Using Rat and Bovine Brain Microvascular Endothelial Cell System (E0755701)

Responsible Division: Neurotoxicology
Collaborating Office: Office of Scientific Coordination
External Partner: University of Arkansas at Little Rock
Objective(s):

1) Evaluate the neurotoxicity of the graphene test materials *in vitro* primary cultures of rat-brain microvascular endothelial cells (rBMEC) and bovine brain microvascular endothelial cells (bBMEC);
2) Determine if *in vitro* exposure to the graphene test materials (a) produce cytotoxicity as measure by XTT, MTT, ROS, RNS alterations, caspase activation and lipid peroxidation, (b) causes alterations in neuroinflammatory markers such as TNF-alpha, IL-1,6 and prostaglandin, and (c) forms 3-nitrotyrosine, an *in vivo* biomarker for oxidative stress. Fluorescine will be used as marker for assessing the permeability of the blood-brain barrier.

Determine, using microarrays, if *in vitro* exposure to these graphene-nanomaterials induce specific genomic/proteomic changes in rBMEC and bBMEC.

PI: Beger, Richard D., Ph.D.

Participation in Data Quality Task Group (DQTG) to Foster Development of Consensus Quality Control (QC) Standards in Metabolomics Data Acquisition (S00787)

Responsible Division: Systems Biology
Objective(s):
The goal of the DQTG efforts would be to move toward validation exercises for analytical QC techniques with multiple international metabolomics groups. By using the greater metabolomics community as the source of analytical QC techniques and criteria information, the DQTG should be able to reach consensus decisions on what samples of QC is used and how those QC samples are evaluated.

PI: Beland, Frederick A., Ph.D.

Distribution of an Adjuvant Containing Squalene and Alpha-Tocopherol in Mice (E0751401)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CBER
Objective(s):
Provide to CBER, experimental pharmacokinetic data in mice for an oil-water adjuvant designated AS03 that will be used to validate an *in silico* Physiologically Based Pharmacokinetic model.

PI: Beland, Frederick A., Ph.D.

Two-Year Carcinogenicity Bioassay of Furan in F344 Rats (E0216801)

Responsible Division: Biochemical Toxicology
Collaborating Division: Bioinformatics and Biostatistics
External Partner: National Toxicology Program
Objective(s):
Determine the dose-response relationship for the carcinogenicity of furan in F344 rats.

PI: Binienda, Zbigniew K., Ph.D.

Assessment of Iron-Oxide Nanoparticle (NP)-Induced Neurotoxicity in Cell Cultures and Whole-Animal Models (E0739401)

Responsible Division: Neurotoxicology
Collaborating FDA Center: CFSAN
Objective(s):

1) Determine if acute or chronic exposure of different sizes of iron-oxide NPs produce specific changes in the mitochondrial function, cell death, and generation of reactive oxygen species in different regions of rat and mice brain using *in vivo* microdialysis.
2) Determine if acute or chronic exposure to iron-oxide NPs produce significant changes in neurotransmitter concentrations in different regions of mice/rat brains using microdialysis.
3) Determine if acute or chronic exposure of different sizes of iron-oxide NPs produce alterations in the brain-free fatty acid levels.
4) Determine if acute or chronic exposure to different sizes of iron-oxide NPs produce changes in lipid peroxidation and/or in antioxidant enzyme activity (catalase, superoxide dismutase, glutathione peroxidase) and glutathione levels in mice and rat brains.
5) Determine if acute or chronic exposure of different sizes of iron-oxide NPs produce selective pattern of deposition and damage in different regions of rat and mice brain using *in vivo* MRI.

PI: Boudreau, Mary D., Ph.D.

13-Week Study To Determine the Pathogenesis of the Whole-Leaf Extract of the *Aloe vera* in the Cecum and Large Intestine of the F-344 Rat (E0218201)

Responsible Division: Biochemical Toxicology
External Partner: National Toxicology Program
Objective(s):

1) Determine the fraction(s) of the *Aloe vera* whole-leaf extract responsible for the non-neoplastic lesions observed in the 13-week study and the carcinogenic effects observed in the two-year bioassay.
2) Evaluate whether other extracts of the *Aloe vera* plant, including *Aloe* gel of the inner parenchyma leaf tissue and *Aloe* decolorized whole-leaf extract, an extract of the whole leaf of *Aloe vera* but treated subsequently with activated carbon, exert similar effects in the rat large intestine.
3) Examine whether Senna, a dietary supplement with components similar to those found in *Aloe vera*, exerts comparable effects in the rat intestine when administered in the drinking water.

PI: Boudreau, Mary D., Ph.D.

A 13-Week Range-Finding Phototoxicity Study To Evaluate the Responses of SKH-1 Hairless Mice to Retinyl Palmitate When Incorporated into a Vehicle Cream Containing Butylated Hydroxytoluene (BHT) and Isopropanol (IPA) (E0219301)

Responsible Division: Biochemical Toxicology
Collaborating Division/Office: Bioinformatics and Biostatistics, Office of Scientific Coordination
Objective(s):

1) Determine whether or not the 10% filter in the vehicle cream composed of mixture of BHT, IPA, and water induces changes in the appearance of skin of SKH-1 mice when compared to the 10% water-only filler in the control cream in the presence and absence of 13.70 mJ CIE/cm2.
2) Evaluate the dose-response effects of retinyl palmitate in cream on the appearance of skin of SKH-1 mice in both the presence and absence of 13.70 mJ CIE/cm2, when the 10% cream filler is composed of increasing concentrations of retinyl palmitate (0.5-8%) in constant amounts of BHT/IPA and decreasing amounts of water.

PI: Boudreau, Mary D., Ph.D.

A Toxicological Evaluation of Nanoscale Silver Particles in Rodents (E0217001)

Responsible Division: Biochemical Toxicology
Collaborating FDA Centers: CDRH, CFSAN
Objective(s):

1) Evaluate the effect of size of nanoscale silver particles on plasma protein-binding in blood collected from adult rodents using standard analysis methods to estimate the equilibrium association constant and maximum binding capacity.
2) Determine the effects of size and dose of nanoscale silver particles on the pharmacokinetic profiles and bioavailability when administered by the oral and intravenous routes in rats, and determine whether the pharmacokinetics of nanoscale silver are the same as silver acetate.

3) Evaluate the absorption, biodistribution (including the potential to cross the blood-brain barrier), and excretion rates of nanoscale silver particles that differ in size.
4) Investigate the site of particle uptake in the GI tract.

PI: Boudreau, Mary D., Ph.D.

An Evaluation of the Effect of Vehicle Cream on the Photococarcinogenicity of Retinyl Palmitate in SKH-1 Mice (E0218501)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CFSAN
Objective(s):

1) Determine the stability and homogeneity of retinyl palmitate in the *Aloe vera* control cream.
2) Evaluate the photococarcinogenicity of retinyl palmitate when incorporated into the *Aloe vera* control cream applied to the skins of SKH-1 mice in the absence and presence of simulated solar light (SSL).
3) Determine the photococarcinogenicity of disopropyl adipate as the filler ingredient in the *Aloe vera* control cream in the absence and presence of SSL.

PI: Cao, Xuefei, Ph.D.

Dose-Response Genotoxicity of Ethylmethane Sulfonate (EMS) in Mice Using the Pig-a and Transgenic gpt Delta Assays (E0739001)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):

1) Use sensitive genotoxicity endpoints with low background frequencies to increase the sensitivity of the assays for detecting low-dose effects.
2) Measure genotoxicity using a design to detect the maximum responses.
3) Measure the effects of EMS exposure in neonatal as well as adult animals.
4) Measure genotoxicity in the major target tissues for EMS carcinogenicity.

PI: Cao, Xuefei, Ph.D.

ADDENDUM to E0739001: Dose-Response Genotoxicity of Ethylmethane Sulfonate (EMS) in Mice using the Pig-a and Transgenic gpt Delta Assays (E0739011)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):
Addendum submitted to modify experiment #3 on the original study which involves recalculating numbers of animals needed, replacing some mice lost due to false-starts and adding two dose groups to the experiment in order to fill in a gap in dosing and to provide a positive control.

PI: Cao, Xuefei, Ph.D.

Evaluating the Toxicity and Inflammation Produced by Cigarette Smoke Using Human *In vitro* Airway Models (E0754901)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Divisions: Biochemical Toxicology, Sytems Biology
Collaborating FDA Center: CTP
Objective(s):

1) Evaluate the toxicological effects of cigarette smoke.

2) Identify the most informative intracellular molecular biomarkers for cigarette smoke toxicity. Cigarette smoke-induced changes in mRNA and miRNA expression and DNA methylation status will be assessed in tissue samples.
3) Identify extracellular molecular biomarkers for cigarette smoke toxicity. Cigarette-smoke-induced changes in miRNA expression will be evaluated by analysis of basal medium and secreted mucus.

PI: Cerniglia, Carl E., Ph.D.

Impact of Melamine on Human Intestinal Microbiota: Does the Human Intestinal Microbiota have the Enzymatic Capacity to Metabolize Melamine to Cyanuric Acid? (P00730)

Responsible Division: Microbiology
Collaborating Division: Biochemical Toxicology
Collaborating FDA Center: CVM
Objective(s):

1) Determine if melamine impacts the population dynamics of the human intestinal microbiota.
2) Determine if the human intestinal microbiota metabolizes melamine to cyanuric acid.

PI: Chen, Huizhong, Ph.D.

Evaluation of Product and Physiologic Variables Influencing Smokeless Tobacco Toxicity (E0747201)

Responsible Division: Microbiology
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology
Collaboring FDA Center: CTP
Objective(s):

1) Determine the effect of smokeless tobacco on oral bacterial ecology.
2) Assay and compare the toxicity and genotoxicity of smokeless tobacco before and after metabolism by oral bacteria.
3) Demonstrate the metabolism of tobacco-specific compounds, N-nitrosamines by oral bacteria.
4) Evaluate the relationship of smokeless tobacco on the antibiotics resistance of oral bacteria.

PI: Chen, James J., Ph.D.

Application of Biometrical Procedures for National Toxicology Program (NTP) Projects (S00175)

Responsible Division: Bioinformatics and Biostatistics
Objective(s):
In response to requests from NCTR scientists, modify and/or apply statistical techniques to the design, conduct, analysis, and interpretation of NTP studies to identify and assess the cancer and noncancer risks of potentially toxic substances.

PI: Delclos, Kenneth B., Ph.D.

Evaluation of Molecular, Morphological, and Functional Endpoints in NCTR Sprague-Dawley Rats Treated with Bisphenol A (BPA) Administered by Gavage to Sprague-Dawley Rats from Gestational Day 6 Until Birth and Directly to Pups from Postnatal Day (PND)-1; Continuous and Stop Dose (PND-21) Exposures (E0219101)

Responsible Division: Biochemical Toxicology
Collaborating Office: Office of Scientific Coordination
External Partner: National Toxicology Program
Objective(s):
Evaluate a range of molecular, morphological, and functional endpoints

in rats dosed orally with a wide range of BPA doses in a chronic toxicology study. Determine if any effects observed are predictive of long-term toxic effects evaluated in the companion chronic toxicology study or reveal potential effects undetected by standard toxicological evaluations.

PI: Delclos, Kenneth B., Ph.D.

Two-Year Chronic Toxicology Study of Bisphenol A (BPA) Administered by Gavage to Sprague-Dawley Rats from Gestational Day-6 until Birth and Directly to Pups from Post-Natal Day (PND)-1, Continuous and Stop Dose (PND-21) Exposures (E0219001)

Responsible Division: Biochemical Toxicology
Collaborating Office: Office of Scientific Coordination
External Partner: National Toxicology Program
Objective(s):
Characterize the long-term toxicity of orally administered BPA, including developmental exposure, in the NCTR Sprague-Dawley rat over a broad dose range. In addition, animals generated in this study will be assigned to separate protocols for assessment of a range of molecular, morphological, and functional endpoints to determine if these endpoints are predictive of long-term toxic effects or reveal potential effects undetected by standard toxicological evaluations.

PI: Doerge, Daniel R., Ph.D.

Human Biomonitoring for Bisphenol A (BPA)(E0743101)

Responsible Division: Biochemical Toxicology
Objective(s):

1) Develop and implement sensitive and selective analytical methodology to measure BPA from blood and urine samples from children and adults with known exposures.
2) Integrate human biomonitoring data with pharmacokinetic data from animals and humans to produce a physiologically based pharmacokinetic model for BPA to empower FDA to reach science-based decisions about risks, particularly to children and fetuses, from medical devices, food contact materials, and other environmental exposures.

PI: Doerge, Daniel R., Ph.D.

Human Biomonitoring for Exposure to Bisphenol A (BPA) and Potential Replacement Products (E0747101)

Responsible Division: Biochemical Toxicology
External Partner: National Institute of Environmental Health Sciences
Objective(s):

1) Provide human biomonitoring data for BPA and its structural analogs that are potential replacement products in adults exposed occupationally to thermal-paper cash register receipts. The routes of administration (dermal/oral) are likely key determinants of internal exposure to the active unconjugated form of BPA and/or possible replacements. These data will be used for physiologically based pharmacokinetic modeling along with existing pharmacokinetic data from experimental animals and humans.
2) Provide estimates of concentrations of active BPA aglycone in potential target tissues of developing fetuses and children for BPA and/or structural analogs from all possible exposures,

particularly from food and medical devices, so FDA can make science-based decisions on risks from BPA and possible replacement products.

PI: Doerge, Daniel R., Ph.D.

Human Pharmacokinetics of Bisphenol A (BPA) (E0750001)

Responsible Division: Biochemical Toxicology

Objective(s):

1) Measure deuterated BPA from blood and urine samples from adult humans after a single oral dose of 100 µg/kg bw in order to resolve uncertainty regarding human metabolism, pharmacokinetics (PK), and mass balance of BPA.
2) Integrate these new human PK and mass-balance data with the PK data from experimental animal models (e.g., mouse, rat, and monkey) along with human urinary biomonitoring data to refine a physiologically based pharmacokinetic model for BPA.

PI: Doerge, Daniel R., Ph.D.

Human Studies of Isoflavone Safety and Efficacy (S00607)

Responsible Division: Biochemical Toxicology

External Partners: University of Miami, Wayne State University

Objective(s):

Conduct bioanalytical analysis of soy isoflavones (and metabolites) in support of clinical trials at the University of Miami and Wayne State University.

PI: Doerge, Daniel R., Ph.D.

Phytoestrogens and Aging: Dose, Timing, and Tissue (E0721001)

Responsible Division: Biochemical Toxicology

Objective(s):

Evaluate the potential benefits or detrimental effects of dietary phytoestrogens on breast cancer progression, adipose tissue, and the brain, using well-established laboratory animal models.

PI: Doerge, Daniel R., Ph.D.

ADDENDUM to E0721001: Phytoestrogens and Aging: Dose, Timing, and Tissue (E0721011)

Responsible Division: Biochemical Toxicology

Objective(s):

Determine bioavailability of soy isoflavones in neonatal and adult male monkeys.

PI: Doerge, Daniel R., Ph.D.

ADDENDUM to E0721001: Phytoestrogens and Aging: Dose, Timing, and Tissue (E0721021)

Responsible Division: Biochemical Toxicology

External Partner: University of Illinois (CRADA)

Objective(s):

Continue a line of collaborative research between NCTR and several University of Illinois investigators that has been active since 2004 and supported by a CRADA that was funded by a grant to the University of Illinois from the National Institute on Aging entitled "Phytoestrogens and Aging: Dose, Timing, and Tissue." The focus of the original NCTR protocol and CRADA was the effect of soy isoflavones on aging in which the NCTR group provided LC/MS data to describe pharmacokinetics and metabolism from animal models developed at the University of Illinois.

PI: Fang, Jia-Long, Ph.D.

Mechanistic Study on the Disruption of Thyroid Hormone Homeostasis Resulting from Sub-Chronic Dermal Exposure of Triclosan to Mice (E0752901)

Responsible Division: Biochemical Toxicology
Objective(s):
Examine the mechanisms underlying the effects of triclosan on thyroid hormone homeostasis in female B6C3F1 mice.

PI: Fang, Jia-long, Ph.D.

Two-Year Dermal Carcinogenicity Bioassay of Triclosan in B6C3F1 Mice (E0219401)

Responsible Division: Biochemical Toxicology
Collaborating Division/Office: Bioinformatics and Biostatistics, Office of Scientific Coordination
External Partner: National Toxicology Program
Objective(s):
Evaluate the chronic toxicity/carcinogenicity of triclosan administered dermally to mice for 104 weeks.

PI: Ferguson, Sherry A., Ph.D.

Effects of Bisphenol A (BPA) Treatment on Hippocampal NMDA Receptors and Estrogen Receptors (ERs) in Rat Brain (E0750801)

Responsible Division: Neurotoxicology
Objective(s):
Use Western blot and immunohistochemistry techniques to determine expression levels of hippocampal NMDA receptor subunits and the estrogen receptor beta in weanling and adult male and female rats developmentally treated with various doses of BPA or EE2.

PI: Ferguson, Sherry A., Ph.D.

Methylphenidate (Ritalin) Exposure During Pregnancy: Assessment of Neurotoxicity in Offspring (E0731801)

Responsible Division: Neurotoxicology
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology, Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):
Quantify the neurobehavioral toxicity associated with pre- and early postnatal treatment with methylphenidate in rats.

PI: Ferguson, Sherry A., Ph.D.

Neurobehavioral Effects of Bisphenol A Across Age and Sex (E0219201)

Responsible Division: Neurotoxicology
External Partner: National Toxicology Program
Objective(s):
Advance development of rapid detection technologies and testing platforms in the area of food safety, biosecurity, food biodefense, and bioterrorism.

PI: Ferguson, Sherry A., Ph.D.

Training for Bisphenol A (BPA) Studies (P00706)

Responsible Division: Neurotoxicology
Collaborating Divisions: Biochemical Toxicology, Bioinformatics and Biostatistics
Objective(s):
Develop the skills and techniques, such as complex behavioral assessments and quantitative volumetric analysis of sexually dimorphic brain regions to conduct subsequent studies of

developmental treatment with BPA.

PI: Fisher, Jeffrey W., Ph.D.

PBPK Models for Bisphenol A (BPA) (E0742601)

Responsible Division: Biochemical Toxicology
Collaborating Division: Neurotoxicology
Objective(s):

1) Create physiologically based pharmacokinetic (PBPK) models for BPA in mouse, rat, and rhesus nonhuman primate of adult, neonatal, pregnant (mother and fetus), and lactating (mother and neonate) laboratory animals. These models will be used to calculate internal measures of dose for both active and inactive forms of BPA.
2) Create human PBPK models for BPA (adult, child, pregnant mother and fetus, and lactating mom and infant) using data from the nonhuman primate, mouse, and rat, and limited human information from literature. The human suite of models will be used to extrapolate the internal toxic doses of BPA in laboratory animals to humans. The PBPK models will also be used to extrapolate dosimetry from regions of observation to low levels of exposure to BPA for which no experimental data exist.
   Interpret biomonitoring data for BPA in urine and blood.

PI: Foley, Steven L., Ph.D.

Characterization of Plasmid-Associated Antimicrobial Resistance in *Salmonella enterica* Serovars Associated with Poultry and Human Infections (E0733501)

Responsible Division: Microbiology
Collaborating Office: Office of the Center Director/OR
Collaborating FDA Center: CVM
Objective(s):

1) Identify and understand the genetic mechanisms associated with plasmids that facilitate the spread and persistence of virulence and multidrug resistance in *Salmonella* from poultry- and egg-associated serovars.
2) Sequence the plasmids from multidrug-resistant *S. enterica* serovar *Enteritidis, Heidelberg*, and *Typhimurium* strains to identify genes likely associated with virulence and antimicrobial resistance.
3) Determine the relative selective potential of antimicrobial agents to trigger the dissemination of antimicrobial-resistance and virulence factors to susceptible *Salmonella.*
4) Determine the contribution of plasmids transferred via conjugation to virulence in *Salmonella* strains.

PI: George, Nysia, Ph.D.

QT Interval Correction via Mixed-Effects Modeling (E0734601)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):
Develop an appropriate non-linear mixed effects pharmacokinetic model in order to examine the effects of bitter orange/synephrine extract on electrocardiography behavior in a National Toxicology Program study.

PI: Goodwin, Amy K., Ph.D.

Aspects of Nicotine Self-Administration in the Nonhuman Primate (E0753701)

Responsible Division: Neurotoxicology
Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination

Collaborating FDA Center: CTP
Objective(s):

1) Set up a surgical suite for implantation of intravenous (IV) catheters in squirrel monkeys and a nonhuman primate behavioral pharmacology laboratory for conducting the self-administration studies, drug mixing, and related tasks.
2) Purchase, quarantine, and habituate 12 early adolescent male squirrel monkeys and 12 adult male squirrel monkeys.
3) Compare the acquisition and maintenance of nicotine self-administration across decreasing doses in adolescent and adult squirrel monkeys.
4) Investigate and compare the abuse liability of the non-nicotine tobacco product constituents myosmine and anatabine in squirrel monkeys using a substitution procedure.
5) Investigate and compare the effects of the non-nicotine tobacco product constituents myosmine and anatabine on responding for nicotine in squirrel monkeys.
6) Describe the pharmacokinetics of IV self-administered nicotine and other tobacco constituents (myosmine and anatabine).
7) Describe and compare alterations in dopamine levels in the midbrain associated with IV-administered nicotine and other tobacco constituents (myosmine and anatabine).

PI: Gough, Bobby J., Ph.D.

The Impact of a Glial Modulator (PPF) on Methamphetamine (METH)-Induced Dopamine (DA) Dynamics: A Microdialysis Study in Rats and Mice (E0743301)

Responsible Division: Neurotoxicology
Objective(s):

1) Simultaneously measure DA and its metabolite levels in the caudate nucleus of rats and mice using dual online injection.
2) Determine effect PPF will have on METH-evoked DA levels.
3) Determine the protective nature of PPF against METH-induced neurotoxicity in both species. Results of pilot study will indicate possible future studies.
4) Strengthen FDA abilities in microdialysis and further validate the use of mice in neurochemical studies.

PI: Gu, Qiang, Ph.D.

Proteomic Assessment of the Cytotoxic Effects of Nanoparticles (NPs) on the Blood-Brain Barrier (BBB) (E0746001)

Responsible Division: Neurotoxicology
Collaborating Divisions/Office: Biochemical Toxicology, Systems Biology, Office of Scientific Coordination
Objective(s):

1) Describe alterations in expression and/or phosphorylation of proteins that are involved in apoptosis, inflammation, oxidative stress, and tumor-genesis signaling pathways in the cells that form BBB following NP exposure using cutting-edge proteomic approaches.
2) Proteomic changes will also be correlated with conventional cytotoxicity and BBB permeability assays. Additional nanoparticles may also be studied if early findings warrant.

PI: Guo, Lei, Ph.D.

Develop Methods for the Evaluation of Smokeless Tobacco-Associated Carcinogenesis (E0748801)

Responsible Division: Biochemical Toxicology
Collaborating Division: Microbiology
Collaborating FDA Center: CTP

Objective(s):

1) Evaluate and compare the carcinogenic activity of smokeless tobacco products.
2) Investigate animal models for comparing and evaluating carcinogenic activities (especially oral-cavity tumor induction) of smokeless tobacco products.
3) Test the hypothesis that tobacco-specific N-nitrosamines (TSNA) are major contributors to carcinogenic activity of smokeless tobacco.
   a. Determine and quantify the major carcinogenic alkaloid-derived TSNA (NNK and NNN) in each product.
   b. Detect and quantify NNK-and NNN-derived DNA adducts in various samples, such as liver, pancreas, blood, and oral tissue, collected from animals administered NNK, NNN, or smokeless tobacco.
4) Determine gene expression and DNA methylation profiles at whole genome level and for specific pathways, such as DNA damage/repair, for biomarker discovery and mechanism elucidation.
5) Determine effect of smokeless tobacco products or TSNA (chemical in smokeless tobacco) on oral microbiota of the animals.

PI: Guo, Lei, Ph.D.

Study of Drug-Induced Liver Toxicity Using State-of-the-Art *In vitro* Liver Models, Including Primary Rat and Mouse Hepatocytes and Stem Cells (E0732101)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Genetic and Molecular Toxicology, Systems Biology
Objective(s):

1) Obtain signature-gene and protein-expression patterns of each cell type for comparison to toxin-induced changes.
2) Determine the contribution of each cell type to overall liver toxicity from agent exposure once these isolated cell types are reliably available.
3) Provide training to give confidence in the integrity of liver cells following perfusion, separation, and culture of the liver cells.

PI: Guo, Xiaoqing, Ph.D.

Development of Methods To Expose Cells in Culture to Volatile Chemicals (E0754301)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination
Collaborating FDA Center: CTP
Objective(s):

1) Develop and demonstrate the reproducibility of a cell culture exposure protocol for volatile test articles by:
   a. Developing a test procedure for the Mouse Lymphoma Assay utilizing suspension cells in culture, the CH Technologies Jaeger-Baumgartner 30-Port Cigarette Smoking Machine, VitroCell exposure chambers, and whole cigarette smoke as the test article.
   b. Demonstrating the reproducibility of the exposure conditions for the MLA by conducting exposures with different concentrations of whole smoke over a period of six months.

PI: Hammons, George J., Ph.D.

In vitro Analysis of Factors Influencing CYP1A2 Expression as Potential Determinants of Sex and Interindividual Variation: Role of Hormones and Epigenetics (E0739301)

Responsible Division: Biochemical Toxicology
Objective(s):

1) Determine the effect of cigarette smoke condensate (CSC) or I3C on CYP1A2 expression in selected liver and lung cell lines.
2) Determine the effect of combining menthol with CSC.
3) Determine the effect of CSC (with and without menthol) or I3C on DNA methylation and histone modification in CYP1A2 as epigenetic regulatory mechanisms in liver and lung cells.
4) Determine the effect of hormones (eg., estrogen, testosterone, growth hormone (GH)) on CYP1A2 expression as factors underlying sex differences in expression.

PI: Hansen, Deborah K., Ph.D.

Developmental Toxicity of Bitter Orange in Rats (E0214701)

Responsible Division: Systems Biology
Collaborating Divisions: Bioinformatics and Biostatistics, Genetic and Molecular Toxicology, Systems Biology
Collaborating FDA Center: CFSAN
Objective(s):
Determine potential developmental toxicity of synthetic synephrine and citrus aurantium extract in rats.

PI: Hansen, Deborah K., Ph.D.

Effect of Oxybenzone on Embryo/Fetal Development in Sprague-Dawley Rats (Segment II) (E0218701)

Responsible Division: Systems Biology
Collaborating Office/Division: Office of Scientific Coordination, Bioinformatics and Biostatistics
External Partner: National Toxicology Program
Objective(s):

1) Determine the potential developmental toxicity of oxybenzone.
2) Compare the results of a typical Segment I, II, III study with results from a modified one-generation study proposed by the National Toxicology Program.

PI: Hansen, Deborah K., Ph.D.

Effect of Oxybenzone on Fertility and Early Embryonic Development in Sprague-Dawley Rats (Segment I) (E0218601)

Responsible Division: Systems Biology
Collaborating Divisions/Office: Biochemical Toxicology, Bioinformatics and Biostatistics, Office of Scientific Coordination
External Partner: National Toxicology Program
Objective(s):

1) Examine the reproductive toxicity of oxybenzone in male and female rats, focusing specifically on fertility and early embryonic development to implantation.
2) Compare the results of a typical Segment I, II, III study design with results from a modified one-generation study proposed by the National Toxicology Program.

PI: Hansen, Deborah K., Ph.D.

Effect of Oxybenzone on Pre- and Postnatal Development in Sprague-Dawley Rats (Segment III) (E0218801)

Responsible Division: Systems Biology
Collaborating Divisions/Office: Biochemical Toxicology, Bioinformatics and Biostatistics, Office of Scientific Coordination
External Partner: National Toxicology Program
Objective(s):

1) Study pre- and early postnatal development [ICH Guideline S5(R2) 4.1.2] to determine the potential toxicity of oxybenzone to male and female rats.
2) Compare the results of a typical Segment I, II, III study with results from a modified one-generation study proposed by the National Toxicology Program.

PI: Hansen, Deborah K., Ph.D.

Physiological Effects of Bitter Orange in Rats (E0214901)

Responsible Division: Systems Biology
Collaborating Divisions: Genetic and Molecular Toxicology, Biochemical Toxicology, Bioinformatics and Biostatistics
Collaborating FDA Center: CFSAN
Objective(s):
Determine potential physiological effects of synthetic synephrine, as well as an extract from the botanical citrus aurantium alone, and in combination with caffeine in rats.

PI: Harris, Steven D., Ph.D.

Center for Tobacco Products (CTP) Scientific Enclave, Tobacco Constituents Knowledge Base, and Topic Modeling for Tobacco Industry Documents (E0753501)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CTP
Objective(s):
1) Provide CTP an external scientific enclave for collaboration.
2) Provide a chemical-centric knowledge base for the > 8400 tobacco constituents.
3) Develop and validate a topic-mining tool to structure tobacco companies' document submissions along thematic topics to aid knowledge discovery in a regulatory context.

PI: Harris, Steven D., Ph.D.

Scientific Enclave, Knowledge Base, and Topic Data Mining for Tobacco Products (E0749001)

Responsible Division: Bioinformatics and Biostatistics
Collaborating Division: Systems Biology
Collaborating FDA Center: CTP
Objective(s):
1) Develop a scientific enclave housing software platforms for collaborative information and data exchange between FDA and collaborators both within and outside of HHS.
2) Develop a Tobacco Constituents Knowledge Base (TCKB).
Develop a scientific data-mining algorithm for textual data on tobacco products.

PI: Heflich, Robert H., Ph.D.

ADDENDUM to E0739001: Dose-Response Genotoxicity of Ethylmethane Sulfonate (EMS) in Mice using the Pig-a and Transgenic gpt Delta Assays (E0739021)

Responsible Division: Genetic and Molecular Toxicology
Objective(s):
Replace the dams used in a previous experiment so we can repeat the neonatal dose-response study in Experiment 3. This experiment was not conducted properly due to a failure of the dosing pump that was not noticed until several daily doses had been administered, making data from this experiment unreliable.

PI: Heflich, Robert H., Ph.D.

Evaluating the Toxicity of Tobacco Products Using *In vitro* 3-D Tissue Models (E0746801)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Systems Biology
Collaborating FDA Center: CTP
Objective(s):

1) Identify the available engineered-tissue models that may be applicable to evaluating the toxicity of tobacco products and ascertain their known characteristics.
2) Conduct preliminary studies to verify important characteristics of the models—when necessary:
   a. Develop data on important baseline characteristics of the models.
   b. Adopt methods to measure tissue toxicity to the models.
   c. Gain experience working with the models.
   d. Identify appropriate positive controls for the major toxicity endpoints.
3) Utilize the most applicable models and endpoints to assess the toxicity of a series of cigarette smoke condensates predicted to differ in their toxicity.

PI: Hiranita,Takato, Ph.D.

Assessment of Effects of Tobacco Product Constituents on Extracellular Dopamine Levels in the Nucleus Accumbens in Rats (E0753801)

Responsible Division: Neurotoxicology
Collaborating FDA Center: CTP
Objective(s):

1) Set up a surgical suite [for implantation of intravenous (IV) catheters and intracranial probes in rodents] and a rodent neurochemical and behavioral pharmacology laboratory [for brain microdialysis/locomotor activity (LMA) studies].
2) Assess the capacity of IV injections of S(-)-nicotine and non-nicotine tobacco product constituents (anatabine, harmane, myosmine, norharmane and their vehicles) by themselves to alter:
   a. extracellular levels of dopamine (DA) in the nucleus acumens shell (NAS)
   b. concurrent spontaneous LMA in drug naïve rats.
3) Assess the capacity of IV injections of the non-nicotine tobacco product constituents anatabine, harmane, myosmine, norharmane and their vehicles to alter S(-)-nicotine-stimulated:
   a. extracellular levels of DA in the NAS
   b. spontaneous LMA in drug naïve rats.

PI: Hong, Huixiao, Ph.D.

High-Throughput Screening Tobacco Constituents for Addiction Potential Using Docking of Nicotinic Acetylcholine Receptors (E0754801)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CTP
Objective(s):

Develop *in silico* models for screening chemicals in tobacco products and smoke that have potential to cause addiction. More specifically, docking analyses will be conducted on all tobacco constituents using three-dimensional (3D) structures of '4ß2 and '7 of which the ligand binding sites will be modeled by using all crystal structures of the complexes of nAChRs bound with ligands that are available in

the PDB (Protein Data Bank). The docking results then will be used for the purpose of predicting addiction potential of the more than 8000 chemicals that have been identified in tobacco products to assist the FDA regulatory decision making or to help the design of follow-up experiments for identifying addictive tobacco constituents.

PI: Howard, Paul C., Ph.D.

ADDENDUM to E0710501: Methodology for Safety Testing of Pigments Used for Tattooing, Including Permanent Make-up (E0710521)

Responsible Office: Office of Scientific Coordination
Collaborating Division: Biochemical Toxicology
Objective(s):
Additional experiments requested to extend the results of E0710501 experiments already completed and underway.

PI: Howard, Paul C., Ph.D.

CTP Prep Studies on Inhalation Toxicity (P00753)

Responsible Office: Office of Scientific Coordination
Collaborating Division: Biochemical Toxicology
Collaborating FDA Center: CTP
Objective(s):

1) Conduct literature review on inhalation toxicology studies conducted with NNK (a nitrosamine present in tobacco).
2) Conduct literature review on pharmacokinetic studies of NNK following any exposure route.
3) Determine the specifications for an inhalation unit.

PI: Hu, Shu-Chieh, Ph.D.

13-Week Nose-Only Inhalation Toxicity Study of NNK in Rats (E0753101)

Responsible Office: Office of Scientific Coordination
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology
Collaborating FDA Center: CTP
Objective(s):

1) Set up a surgical suite for implantation of intravenous (IV) catheters in squirrel monkeys and a nonhuman primate behavioral pharmacology laboratory for conducting the self-administration studies, drug mixing, and related tasks.
2) Purchase, quarantine, and habituate 12 adolescent squirrel monkeys and 12 adult male squirrel monkeys.
3) Compare the acquisition and maintenance of nicotine self-administration across decreasing doses in adolescents and adults.
4) Investigate and compare the abuse liability of the non-nicotine tobacco product constituents myosmine and anatabine in squirrel nonhuman primates using a substitution procedure.
5) Investigate and compare the effects of the non-nicotine tobacco product constituents myosmine and anatabine on responding for nicotine in squirrel monkeys.
6) Describe the pharmacokinetics of IV self-administered nicotine and other tobacco constituents (myosmine and anatabine).
7) Describe and compare alterations in dopamine levels in the midbrain associated with IV-administered nicotine and other tobacco constituents (myosmine and anatabine).

PI: Hu, Shu-Chieh, Ph.D.

14-Day Nose-Only Inhalation Toxicity Study of NNK in Rats (E0753401)

Responsible Office: Office of Scientific Coordination
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology
Collaborating FDA Center: CTP
Objective(s):
Evaluate the biological responses in rats following nose-only inhalation exposure of NNK for 14 days.

PI: Hu, Shu-Chieh, Ph.D.

Pharmacokinetic Analysis of NNK in Sprague-Dawley Rats (E0752501)

Responsible Office: Office of Scientific Coordination
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology
Collaborating FDA Center: CTP
Objective(s):
Evaluate the pharmacokinetic parameters of NNK in rats following a single-dose administration of test substance via intraperitoneal injection, nose-only inhalation exposure, and oral gavage, respectively.

PI: Hu, Shu-Chieh, Ph.D.

Support of CTP/NCTR Inhalation Toxicology Core Facility (S00785)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Center: CTP
Objective(s):
The objective for the CTP/NCTR Inhalation Toxicology Core Facility (Inhalation Core) are to conduct inhalation toxicology studies on behalf of the FDA Center for Tobacco Producs (CTP). The intent of this project/support is to account for the inter-study efforts that are required for the inter-study processing and validation of components of the equipment used in the Inhalation Core.

PI: Kanungo, Jyotshnabala, Ph.D.

Developmental Neurotoxicity Assessment of NMDA Receptor Antagonists in Zebrafish (E0752801)

Responsible Division: Neurotoxicology
Objective(s):

1) Assess the effects on Rohon-Beard sensory neurons of WT zebrafish embryos exposed to NMDA receptor antagonists (MK-801, dextromethorphan, ketamine and sevoflurane).
2) Assess their effects on the primary and secondary motor neurons and their axons using hb9:GFP transgenic embryos.
3) Post-exposure, wash-out experiments will be pursued to determine the effects of these drugs on the nervous system.
4) Determine estradiol-17' levels in control and treated embryos. Changes in gene expression for the two CYP aromatases/estrogen synthases (brain aromatase cyp19a1b and gonadal aromatase cyp19a1a) will be quantified using qPCR.
5) Perform ELISA to quantify the protein level.
6) Assess phenotype-based cell signaling mechanisms (MAPK, etc.) and neuron development-specific gene (Notch, Gli, Ngn1, NeuroD) expression.
7) Utilize neurotoxicity pathway-focused gene expression arrays (SABioscience) to demonstrate potential genotype-phenotype correlations.
8) Reversal of noted adverse effects of

these compounds on neurons will be attempted, particularly by treatment with acetyl L-carnitine.

PI: Kanungo, Jyotshnabala, Ph.D.

Effect of Pediatric Anesthetics on Zebrafish Embryos: Neurotoxicity vs. Gene Expression Changes and Neuronal Kinase (Cdk5) as a Mediator of Toxicity (E0736301)

Responsible Division: Neurotoxicology
Objective(s):

1) Determine if ketamine will have neurotoxic effects (on neurogenesis and axonogenesis) in zebrafish.
2) Determine if the window of such effects varies between early and late differentiating neurons (sensory and motor neurons, respectively).

PI: Khan, Saeed A., Ph.D.

Antimicrobial Properties of Zinc Oxide (ZnO) and Titanium Oxide (TiO2) Nanoparticles (NPs) Against Multidrug-Resistant *Staphylococcus* and *Enterococcus* spp. and Their Cytotoxic and Genotoxic Potential in Bacteria and Normal Human Epidermal Keratinocytes (NHEK) and Primary Intestinal Cells (E0751501)

Responsible Division: Microbiology
Collaborating Division/Office: Systems Biology, Office of Scientific Coordination
Objective(s):

1) Study the mechanism of antimicrobial properties of TiO2 and ZnO Nanoparticles (NPs) in multidrug-resistant *Staphylococcal* and *Enterococcal* spp.
2) Study synergy between NPs and antibiotics.
3) Evaluate the cytotoxic and genotoxic potential of NPs in bacterial, and NHEK and primary intestinal-cell lines.
4) Study the transcriptomic gene expression in NHEK and intestinal-cell lines.

PI: Khare, Sangeeta, Ph.D.

Assessment of Size- and Shape-Dependent Toxicity of Silver Nanoparticles as Measured by Changes in the Permeability at the Gastrointestinal Surface (E0750601)

Responsible Division: Microbiology
Collaborating Office: Office of Scientific Coordination
Objective(s):

1) Determine the effect of nanomaterials on the permeability of intestinal epithelial cells and ileal mucosa.
2) Assess tocixity of silver nanoparticles as measured by changes in the expression of genes involved in the epithelial integrity of polarized epithelial cells and ileal mucosa.

PI: Khare, Sangeeta, Ph.D.

Graphene-Induced Toxicity on the Population of Intestinal Microbiota and Gut-Associated Immune Response (E0754701)

Responsible Division: Microbiology
Collaborating Office: Office of Scientific Coordination
Objective(s):

Evaluate the effects of graphene on the gastrointestinal homeostasis. To accomplish this goal we have subdivided the study in two phases. Phase one of the study will include *in vitro* assessment of effect of graphene; whereas phase two will include the *in vivo* study. The objective of these phases will be as follow:

Phase One (*in vitro* assessment):

1) Effect of graphene on the representative species of

intestinal bacteria. The effect of graphene will be examined in a time- and dose-dependent manner.

2) Effect of graphene on the permeability of polarized epithelial cells. Graphene will be tested to assess the permeability and integrity of *in vitro*-cultured intestinal epithelial cell.

Phase Two (*in vivo* assessment):

1) Evaluate the effects of the graphene on the intestinal commensal microbiota in the orally gavaged rats. Perform a comprehensive culture-independent phylogenetic analysis of intestinal mucosa-associated microbes to analyze the effect of graphene on microbiome present in intestine in the representative from rat intestinal mucosa and feces.
2) Delineate the interaction of orally gavaged graphene at the gastrointestinal surface by assessing the gut-associated immune responses.
3) Measure by real-time polymerase chain reaction the expression of genes involved in the host innate immune response (proinflammatory and anti-inflammatory genes).

PI: Kim, Seong-Jae, Ph.D.

Evaluate the Impact of Deepwater Horizon Oil-Contaminated Gulf Seafood Residues in Edible Tissues on the Human Intestinal Microbiota of the Consumer (E0742801)

Responsible Division: Microbiology
Collaborating FDA Center: CFSAN
Objective(s):

1) Determine whether metabolized polycyclic-aromatic hydrocarbon (PAH) residues in edible tissues of Deepwater Horizon oil-contaminated seafood adversely affect the human intestinal microbiota.
2) Determine if the human intestinal microbiota metabolize PAHs that are toxic components of Deepwater Horizon oil.
3) Identify, characterize, and determine the toxicity of PAH metabolites generated from degradation by human intestinal microbiota.

PI: Leakey, Julian E., Ph .D.

Physiologically-Based Pharmacokinetic (PBPK) Modeling of Nanomedicine; Building Clinically Relevant Standards for FDA-Regulated Nanoparticulate Drug Products (E0755401)

Responsible Office: Office of Scientific Coordination
Collaborating Division: Microbiology
Collaborating FDA Centers/Office: CDER, CVM, ORA
Objective(s):

1) Determine *in vivo* liposomal doxorubicin-release kinetics in individual tissues and blood stream by PBPK modeling.
2) Establish quantitative physicochemical property (liposomal size and content of ammonium sulfate)-biodistribution relationships of liposomal doxorubicin products by PBPK Modeling.
3) Extrapolate the PBPK model to rats and humans.
   Develop a whole-body PBPK model to describe and simulate the biodistribution of liposomal vesicles and doxorubicin.

PI: Leakey, Julian E., Ph.D.

RANGE-FINDING ONLY—Studies of Usnic Acid and Usnea Barbata Herb in Fischer 344 Rats and B6C3F1 Mice (E0215911)

Responsible Office: Office of Scientific Coordination
Collaborating Divisions: Biochemical Toxicology, Systems Biology
Collaborating FDA Center: CFSAN
Objective(s):
Establish appropriate doses of usnic acid and Usnea barbata preparations, administered in feed, in male and female Fischer 344 rats and B6C3f1 mice, for use in subsequent subchronic and chronic studies.

PI: Manjanatha, Mugimane, Ph.D.

Validation of Recently Established gpt-Delta Mice at NCTR (E0740201)

Responsible Division: Genetic and Molecular Toxicology
Objective(s):
1) Characterize recovered mutants from target tissues for generation of gpt and spi mutational spectra.
2) Analyze RBC for Pig-A mutant frequencies and erythrocytes for micronucleus frequencies, respectively.

PI: Mattes, William B., Ph.D.

Understanding and Predicting Immune-Mediated Idiosyncratic Drug Reactions (IDR): Molecular Modeling of Interactions Between Drugs, Polymorphic HLA Proteins, and T-Cell Receptors (E0739501)

Responsible Division: Systems Biology
Collaborating Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):
Apply molecular modeling approaches to better understand the underlying mechanisms of existing drug-HLA combinations known to cause immune-mediated IDRs. Prediction models linking IDRs to specific interaction patterns between drugs and patient-specific polymorphic HLA and T-cell receptors may show enhanced predictability. The modeling approach, if proven to work for existing IDR cases, may be applicable to new drug-patient combinations.

PI: McDaniel, Lea Patrice

Procurement of Equipment to Expose Cell, Tissue, and Bacterial Cultures to Tobacco Smoke for Protocols E0745901 and E0746801 (E0751301)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Office: Regulatory Compliance and Risk Management
Collaborating FDA Center: CTP
Objective(s):
1) Determine the technical specifications/requirements for the exposure modules required for the:
   a. Ames test
   b. Mouse Lymphoma Assay utilizing suspension cell cultures
   c. Micronucleus Assay utilizing both monolayer and suspension cell cultures
   d. 3D human airway tissue-culture models.
2) Work with FDA's CTP staff and OAGS staff to procure the required modules.
3) Work with NCTR's Regulatory Compliance and Risk Management staff to identify safety- and waste-related issues associated with using the modules.

PI: Meehan, Joseph F., Ph.D.

Support of Collaborative Regulatory Review and Research Projects with FDA-CDER (S00784)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):
Provide assistance in the development and enhancement of regulatory review and research tools at CDER, including enhancements to CDER's Data Analysis and Search Host system, development of a Pediatric Clinical Trials database, enhancement of the FDALabel system, genomics data analysis and review, and additional joint biomedical informatics projects as requested.

PI: Mei, Nan, Ph.D.

Evaluation of the Ability of Standard Genetic Toxicology Assays To Assess the Relative Genotoxic Potential of Cigarette Smoke Condensates (E0745901)

Responsible Division: Genetic and Molecular Toxicology
Collaborating FDA Center: CTP
Objective(s):
1) Optimize short-term assays to evaluate their ability to initially assess the genotoxicity of cigarette smoke condensates. Based on the results from these studies, further research using commercial cigarettes and whole cigarette smoke will be designed.
2) Attain a dynamic potency range adequate to detect reductions of select harmful and potentially harmful constituents by 30, 50, and 70 percent.
3) Develop and validate a quantitative assay or assays to detect statistically significant differences in cigarette-smoke cytotoxicity over a range of biologically relevant concentrations.
4) Evaluate genotoxicity assays to determine their robustness, sensitivity, reproducibility, and accuracy.

PI: Mei, Nan, Ph.D.

Genetic Toxicology Evaluations in Support of FDA Centers for Evaluating Substances for their Genotoxic Potential (S00677)

Responsible Division: Genetic and Molecular Toxicology
Objective(s):
Provide direct research to FDA Product Centers.

PI: Mittelstaedt, Roberta A.

Using Standard Genetic Toxicology Assays To Assess the Genotoxic Potential of Smokeless Tobacco Products (E0752101)

Responsible Division: Genetic and Molecular Toxicology
Collaborating FDA Center: CTP
Objective(s):
Assess the genotoxicity of smokeless tobacco extracts. The test materials will be provided by CTP and consist of five commercial products extracted with three solvents: DMSO, phosphate buffered saline, and artificial saliva.

PI: Nakamura, Noriko, Ph.D.

Effect of Fetal Exposure to Oxybenzone on Reproductive Organs of Postnatal Day (PND)-21 Rats (E0745501)

Responsible Division: Systems Biology
Collaborating Division: Bioinformatics and Biostatistics
Objective(s):
1) Determine if fetal exposure to oxybenzone influences the male and

female reproductive organs of Harlan Sprague Dawley rats on PND-21 by examining morphology of the reproductive organs and mRNA expression of genes related to the endocrine system.
2) Determine if fetal exposure to oxybenzone alters morphology of the testis and ovary of PND-21 rats.
3) Determine if fetal exposure to oxybenzone disrupts steroid biosynthesis of male reproductive organs of PND-21 rats.

PI: Nayak, Rajesh R., Ph.D.

Investigating the Mechanisms of Drug Resistance and Pathogenicity in Clinical *Escherichia Coli* Isolates from Veterinary Sources (E0744901)

Responsible Division: Microbiology
Collaborating FDA Center: CVM
Objective(s):
1) Evaluate the antimicrobial susceptibility profiles in *E. coli* and detect the prevalence of antimicrobial-resistance genes for the resistant phenotypes.
2) Investigate the mutational changes in gyrase and regulatory genes, and assess the role of plasmids and integrons in mediating the drug resistance.
3) Characterize the molecular basis of drug resistance in *E. coli* displaying extended-spectrum beta-lactamase phenotypes.
4) Evaluate the virulence gene profiles of the isolates.

PI: Mei, Nan, Ph.D.

In vitro Genotoxicity of Graphene-Family Nanomaterials Using FDA-Recommended Short-Term Genetic Toxicity Test Battery (E0753301)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Office: Office of Scientific Coordination
Objective(s):
1) Determine whether graphene and its functionalized derivatives are genotoxic using the standard regulatory test battery (the Ames test, the mouse lymphoma assay, and the micronucleus assay).
Compare the results from the three assays and provide insight into mechanisms underlying graphene's genotoxic effects.

PI: Nayak, Rajesh R., Ph.D.

Microbial Genetics of Non-0157:H7 Shiga-Like Toxin Producing *Escherichia Coli* Insulated From Humans and Foods (E0735701)

Responsible Division: Microbiology
Collaborating Division/Office: Biochemical Toxicology, Office of Research
Objective(s):
1) Obtain *E. coli* isolates from clinical, food-related outbreaks and veterinary-diagnostics samples.
2) Map the epidemiological profiles of the isolates for specific genetic markers attributable to the origin of isolates and their phenotypic diversity.
3) Determine the antimicrobial-resistance profiles and potential mechanisms of drug resistance among enterohemorrhagic escherichia coli (EHECs) of various serogroups.
4) Identify the antimicrobial-resistance and virulence-gene determinants in the bacterial isolates that contribute to their pathogenicity.
5) Examine the role of plasmids, if any, in mitigating the transfer of drug resistance.
6) Compare the cytoxicities of selected EHEC strains to cultured RAW264.7

macrophage cells.

7) Evaluate the expression of Shiga-like toxin using *in vitro* enzyme assays.

PI: Parsons, Barbara L. Ph.D.

ADDENDUM to E0722901: Development of a Method To Use *In vivo* Mutagenicity Data to Address the Question as to Whether a Specific Chemical Induces Cancer Via a Mutagenic or a Non-mutagenic Mode-of-Action (MOA)—CRADA with TERA (E0722911)

Responsible Division: Genetic and Molecular Toxicology

Objective(s):

1) Establish culturing conditions for GDL1 cells and characterization of cell growth when two different sera are used in the growth media.
2) Expose GDL1 cells grown in DMEM with both FBS and HS to different doeses of a mutagen, N-ethyl-N-nitrosourea (ENU) and a clastogen, mitomycin C (MMC) for induction of mutations in the gpt, and spi- genes for validation of the cell line for testing vinyl acetate monomer (VAM) and acetaldehyde (AA).
3) Expose GDL1 cells cultured in DMEM with both FBS and HS to different doeses of VAM and AA for cytotoxicity and induction of mutations in the gpt, and spi- genes at doses that induce low, mid, and high cytotoxicity in GDL1 cells.
4) Further characterize several mutants if the induced mutant frequency is statistically significant, by DNA sequencing for VAM and AA-induced mutational profiles.

PI: Parsons, Barbara L., Ph.D.

ADDENDUM to E0722901: Assessment of the Importance of K-RAS Mutation Induction in the Mode-of-Action for Lung-Tumor Development and Implications of K-Ras Mutant Subpopulations in Cancer Therapies (E0722921)

Responsible Division: Genetic and Molecular Toxicology

External Partner: Toxicology Excellence for Risk Assessment

Objective(s):

The basic mode-of-action hypothesis to be tested in this phase of the CRADA will be that vanadium pentoxide (VP) induces lung tumors in mice by causing clonal expansion of pre-existing K-Ras mutations.

PI: Paule, Merle G., Ph.D.

ADDENDUM to E0215101: Developmental Neurotoxicity Assessment of Acrylamide in Rats—Long-Term Studies (E0215111)

Responsible Division: Neurotoxicology

Collaborating Divisions: Biochemical Toxicology, Bioinformatics and Biostatistics, Systems Biology

Objective(s):

Determine the consequences of long-term exposure to acrylamide on a variety of developmental milestones and measures of nervous-system integrity throughout life.

PI: Paule, Merle G., Ph.D.

Establishing Rodent and Nonhuman-Primate Behavioral Pharmacology Laboratories at NCTR: Staffing and Equipment (E0749201)

Responsible Division: Neurotoxicology

Collaborating FDA Center: CTP

Objective(s):

1) Hire experienced Ph.D.-level rat and nonhuman-primate drug self-administration experts and laboratory support personnel.
2) Purchase equipment and supplies to support rat and nonhuman primate nicotine self-administration studies.

PI: Paule, Merle G., Ph.D.

Establishing Rodent and Nonhuman Primate Nicotine Behavioral Pharmacology Laboratories at NCTR: Facilities Renovation (P00757)

Responsible Division: Neurotoxicology
Objective(s):

1) Renovate two rooms to accomodate nonhuman-primate housing and a nicotine self-administration laboratory.
2) Upgrade existing rodent testing rooms to accommodate nicotine.

PI: Paule, Merle G., Ph.D.

Long-Term Consequences of Neonatal Ketamine Anesthesia in Rhesus Monkeys: Extended Cognitive Assessments (E0736401)

Responsible Division: Neurotoxicology
Collaborating Office: Office of Scientific Coordination
Collaborating FDA Center: CDER
Objective(s):

1) Continue monitoring the cognitive capabilities of rhesus nonhuman-primate subjects that were exposed to a single, 24-hour bout of ketamine-induced anesthesia during the first week of life. Data to date indicate that, compared to control animals, ketamine-exposed subjects exhibit significant deficits in several aspects of brain function, including learning, the ability to perform simple visual discriminations, motivation, and speed of psychomotor processing. Continuing these observations will provide valuable information on the ultimate time course and severity of the observed deficits.
2) Extend the functional domains that are being assessed. Performance of a temporal discrimination task (timing task), a counting task, and reversal learning tasks (cognitive flexibility) will be added to the current assessment battery.

PI: Pogribny, Igor P., Ph.D.

Development and Evaluation of a Novel *In vitro* Epigenomic Screening Model System for the Hazard Identification of FDA-Regulated Products (E0755001)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CTP
Objective(s):

1) Determine the dose-dependent *in vitro* genetic and epigenetic effects of compounds regulated by FDA.
2) Characterize the specific epigenetic changes induced *in vitro* by genotoxic and non-genotoxic compounds.
3) Characterize the specific genetic and epigenetic effects of compounds regulated by FDA using an *in vitro* 3-D organotypic liver-culture model system.

PI: Pogribny, Igor P., Ph.D.

Relationship Between Liver Epigenetic Phenotype and Susceptibility to Nonalcoholic Steatohepatitis (NASH)-Induced Hepatocarcinogenesis in Mice (E0735301)

Responsible Division: Biochemical Toxicology
Collaborating Division: Systems Biology
Objective(s):

1) Determine the role of epigenetic dysregulation in the etiology and

pathogenesis of dietary NASH-induced hepatocarcinogenesis in mice.
2) Determine whether or not interstrain-specific susceptibility of mice to NASH-induced hepatocarcinogenesis is associated with differences in individual hepatic epigenetic phenotypes.
3) Determine the role of epigenetic dysregulation in the etiology and pathogenesis of NASH-induced hepatocarcinogenesis in mice induced by tamoxifen administration.
4) Determine if aberrant epigenetic markers can be used as targets for prevention of NASH-induced hepatocarcinogenesis in mice.

PI: Rafii, Fatemeh, Ph.D.

Antimicrobial Susceptibilities of *Clostridium Perfringens* Strains Isolated from Different Sources and Genetic Characterizations of Resistance (E0751601)

Responsible Division: Microbiology
Objective(s):
Provide new insight into the molecular basis for the spread of drug resistance in pathogenic bacteria and determine if antibiotic-resistant environmental *C. perfringens* strains are capable of acting as reservoirs for antibiotic resistance genes for human-restricted antibiotics, which are not used in animals at all.

PI: Sung, Kidon, Ph.D.

Quantification Proteomic, Transcriptomic, and Phenotypic Microarray Analysis of *C. Jejuni* for the Identification of Colonization Factors in Poultry (E0735601)

Responsible Division: Microbiology
Collaborating Division/Office: Systems Biology, Office of Scientific Coordination
Objective(s):
1) Evaluate genomic and phenotypic microarrays, and whole proteomic analyses to compare genes, phenotypes, and proteins from both good and poor *C. Jejuni* chicken colonizers.
2) Investigate functional role of identified colonizing factors by mutant construction, *in vitro* assays, and *in vivo* assays.
3) Identify potential targets for vaccine that will enable us to eliminate the threat of *Campylobacter* infection in chickens.

PI: Tolleson, William H., Ph.D.

Rapid Detection of Ribosome-Inactivating Protein Toxins in Foods (E0736101)

Responsible Division: Biochemical Toxicology
Collaborating Division: Microbiology
Collaborating FDA Center: CFSAN
Objective(s):
Provide robust methods for detecting the biological activity of the potential bioterrorism agents ricin, abrin, and shiga-like toxins, each of which is characterized as a ribosome-inactivating protein toxin, in three selected foods (spinach, apple juice, and milk).

PI: Tong, Weida, Ph.D.

Priority Setting of Harmful and Potentially Harmful Constituents (HPHCs) in Tobacco Smoke Products with Bioinformatics (E0750901)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CTP
Objective(s):
1) Identify and predict the major health endpoints (e.g., cancer, cardiovascular, non-neoplastic respiratory, developmental/reproductive, and

addiction) for tobacco constituents and compounds. The information about these endpoints can then be used for the purpose of priority-setting to assist the decision-making or the design of follow-up experiments.
2) Develop and apply the bioinformatics methods to prioritize the HPHCs using the literature data and public databases.
3) Extend the application to the entire list of the approximately 8,400 tobacco constituents for priority-setting.

PI: Trbojevich, Raul, Ph.D.

Study of Nanoparticles Migration from Food-Contact Nanomaterials: Characterization and Quantification of Silver Nanoparticles in Stimulants (E0736801)

Responsible Division: Biochemical Toxicology
Collaborating Office:
Office of Scientific Coordination
Collaborating FDA Office: ORA
Objective(s):
1) Study migration of nanoparticles from nanocomposites used in food-contact materials.
2) Characterize and quantify silver nanoparticles in food simulants.

PI: Wang, Cheng, Ph.D.

Assessment of Gaseous Anesthetics in the Developing Nonhuman Primate (E0728501)

Responsible Division: Neurotoxicology
Collaborating Division/Office:
Biochemical Toxicology, Office of Scientific Coordination
Objective(s):
1) Evaluate dose-response effects of gaseous anesthetics.
2) Determine if prolonged exposure to either nitrous oxide or isoflurane will result in an increase in neuronal-cell death.
3) Determine if combinations of nitrous oxide and isoflurane will prevent or enhance the other's effects on the developing nonhuman primate.
4) Determine if a relative high dose or prolonged exposure of the developing nonhuman primates to either nitrous oxide or isoflurane—or in combination—will induce long-term behavioral deficits, as well as long-lasting pathological changes.
5) Determine, using noninvasive imaging techniques [High resolution dedicated positron emission tomography (microPET) and magnetic resonance imaging (MRI)], if a high dose or prolonged exposure of the developing nonhuman primates to either nitrous oxide or isoflurane, or in combination, will induce long-lasting pathological changes. MRI will be used to verify pathological evidence and look for volume changes. MicroPET will be used to examine the sensitivity for tracing low picomolar concentrations of radiolabeled molecules, which is useful for studying dynamic imaging in animal models of human diseases.
6) Identify potential underlying mechanisms that could link alteration of mitochondrial function and elevation of reactive oxygen species to gaseous anesthetic-induced neuronal-cell death. L-carnitine will be used to attenuate neurological brain injury associated with mitochondria-related degenerative effects induced by gaseous anesthetics in the developing nonhuman primate.

PI: Wang, Cheng, Ph.D.

The Use of Computed Tomography (CT) Combined with Positron Emission Tomography (microPET) To Evaluate the Neurotoxicity Associated with Pediatric Exposures to the Anesthetics Sevoflurane and Propofol (E0749401)

Responsible Division: Neurotoxicology
Objective(s):

1) Using sevoflurane and propofol as test agents, develop standard operating procedures (SOPs) for the combined use of NCTR's microPET and CT scanners in studies on the neurotoxicity associated with the pediatric use of general anesthetics and sedatives.
2) Ensure that all new SOPs required for subsequent experiments using developing animals have been optimized and that the research staff and support personnel have been thoroughly trained to properly handle radioactive animals and utilize both the microPET and CT scanners.
3) Develop and document specific procedures for using CT scans to localize associated PET images in 3D to specific brain areas and structures so that it will be possible to compare volumes of brain areas/structures among experimental groups.
4) Determine the temporal relationships between anesthetic exposure and tracer signal detection using several newly developed [18F]-labeled compounds including those for labeling peripheral benzodiazepine receptors (activated glia), stem cells, and caspase-3, a marker of apoptosis.
5) Determine whether *in vitro* exposures of embryonic-neural stem cells to sevoflurane and propofol impair cellular proliferation. It is hoped that such observations will provide information about the possible mechanisms underlying the neurotoxicity caused by these agents *in vivo.*

PI: Yang, Xi, Ph.D.

ADDENDUM to E0747701: Use of New Technologies to Develop Biomarkers of Harm for New Tobacco Products (E0744711)

Responsible Division: Systems Biology
Collaborating Divisions/Office: Biochemical Toxicology, Bioinformatics and Biostatistics
Collaborating FDA Product Center: CTP
Objective(s):

1) Assess various omics changes (genomics, metabolomics, and proteomics) in two primary lung-cell types and two cardiac-cell types. At this stage, only tobacco smoke condensate (TSC) will be tested. Once smoking machines (for *in vitro* cell-culture exposure) are purchased and running, whole smoke will be tested and this work will be added to the protocol via an addendum. Two different test cigarettes and smoking simulation conditions will be used.
2) Analyze the omics data to determine if any of the omics changes can be used as biomarkers of harm.

PI: Yang, Xi, Ph.D.

Preliminary Study for Whole Smoke Acute Exposure in Lung Cells (E0755301)

Responsible Division: Systems Biology
Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination
Collaborating FDA Center: CTP
Objective(s):

1) Obtain *in vitro* exposure systems and validate the whole smoke generated by the smoking machine.
2) Optimize whole-smoke exposure conditions in human lung cells. Immortalized human bronchial epithelial cells will be cultured in cell inserts and then exposed to whole smoke using an *in vitro* exposure system.
3) Conduct preliminary whole-smoke concentration-response assessments using gross cytotoxicity assay. Intracellular adenosine triphosphate concentration in cells will be measured and the standard curves of treatment-related cell death will be established.

PI: *Zhang, Xuan, Ph.D.*

ADDENDUM to E0742401: Chronic Methylphenidate Administration in Rhesus Monkeys (E0742411)

Responsible Division: Neurotoxicology
Collaborating Division: Biochemical Toxicology
Objective(s):
Dosing and behavior assessments have been and will continue to be conducted in current protocol on these animals to determine the long-term influence of MPH on learning and behavior.

PI: *Zhang, Xuan, Ph.D.*

PET-CT Imaging Using Translatable Biomarkers for Evaluating the Central Nervous System Effects of Chronic Methylphenidate (MPH) Administration in Rhesus Monkeys (E0742401)

Responsible Division: Neurotoxicology
Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination
Objective(s):
1) Determine if prolonged treatment with MPH induces long-lasting changes in specific neurotransmitter receptors using micro postitron emission tomography (microPET). Computed tomography will be used to localize PET findings and visualize brain structures and volumes.
2) Identify potential neurochemical alterations related to the noted behavioral changes seen in these animals using minimally invasive imaging techniques.

PI: Ahn, Young-Beom, Ph.D.

Exploring Strategies for Resuscitation and Enrichment of *Burkholderia Cepacia* Complex Strains in Pharmaceutical Products (E0749801)

Responsible Division: Microbiology
Collaborating FDA Center: CDER
Collaborating Division/Offices: Systems Biology, Office of Scientific Coordination, Office of Research
Objective(s):

1) Screen and identify strains of *B. cepacia* that are difficult to cultivate from pharmaceutical water.
2) Develop a resuscitative step and enrichment technique for *B. cepacia* complex recovery.
3) Develop methodology to detect *B. cepacia* and its 16 related genomovars.
4) Evaluate the use of modern molecular technologies to identify *B. cepacia* complex.

PI: Ahn, Young-Beom, Ph.D.

Impact of Antimicrobial Residues on the Human Gastrointestinal Tract Microbiota (E0732701)

Responsible Division: Microbiology
Collaborating Division: Biochemical Toxicology
Collaborating FDA Center: CVM
Objective(s):

1) Develop methodology to determine if antimicrobial-agent residues bound to fecal contents are microbiologically active.
2) Evaluate the use of current molecular biology, genomic, and proteomic technologies to determine the impact of antimicrobial-agent residues on the human-intestinal microbiota.
3) Determine the potential of the intestinal microbiota to metabolize antimicrobial residues.

PI: Azevedo, Marli P., Ph.D.

Development of an Infectivity Assay to Detect Human Norovirus from Contaminated Food (E0745801)

Responsible Division: Microbiology
Collaborating FDA Center: CBER
Objective(s):

1) Develop alternative assays to detect infectious norovirus from contaminated food.
2) Gain insights on norovirus *in vitro* replication.

PI: Azevedo, Marli P., Ph.D.

Molecular and Seroepidemiology of Coronavirus and Disease Spectrum in Adults, Children, Domestic Animals, and Wildlife in the U.S. (E0738001)

Responsible Division: Microbiology
Collaborating Office: Office of Research
Collaborating FDA Center: CBER
Objective(s):

1) Investigate the molecular epidemiology of circulating enteric and respiratory human CoV (HCoV) and nonhuman CoV strains.
2) Determine whether there is substantial genetic variability of HCoV in our community and examine geographical genetic variation by comparisons with published findings.
3) Determine the zoonotic potential and health-safety threat of newly emerging CoV by comparing to strains currently circulating in domestic animals and wildlife.
4) Define the seroepidemiology and cross-

reactivity of circulating HCoV with known human and nonhuman CoV.

5) Generate immunobiologicals to develop an immunoassay to detect HCoV antibodies for new strains with high antigenic variation.
6) Use the HCoV-specific antibody ELISA or virus-neutralization assays to define the seroepidemiology of the newly detected viruses in adults and children and estimate their prevalence.

PI: Bearden, Ted

Support of CDER/Office of Counter Terrorism and Emergency Coordination "Animal Model" Project (S00753)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):
Provide support to the Center for Drug Evaluation and Research "Animal Model Database for Medical Countermeasures" in the design and testing of a logical model and a physical database schema derived from that model

PI: Bowyer, John F., Ph.D.

Developing More Complete Genomic and Histological Evaluations of Vascular Damage in the Brain Meninges and Choroid Plexus After Neurotoxic Insult (E0751901)

Responsible Division: Neurotoxicology
Collaborating Divisions/Office:
Biochemical Toxicology, Bioinformatics and Biostatistics
Objective(s):

1) Identify additional biomarkers enabling a further understanding of the functions of the MAV and choroid plexus.
2) Develop better histological methods to evaluate vascular damage in the MAV, choroid plexus, and brain.

Successful completion will enable the application of methods developed in this protocol to be applied to a future protocol that will evaluate how a damaged blood-brain barrier (BBB) interacts with various drugs with respect to increasing/altering their neurotoxicity and whether this interaction further compromises the BBB.

PI: Buzatu, Dan A., Ph.D.

Examination of a Novel Flow Cytometer as a Diagnostic Platform for Rapid Determination of Bacterial Antibiotic Resistance and the Presence of Viruses, Prions, or Parasites in Clinical Samples (E0746901)

Responsible Division: Systems Biology
Collaborating Division/Office:
Microbiology, Office of Scientific Coordination
Collaborating FDA Center: CBER
Objective(s):
Examine a variety of adaptations to the diagnostic method and platform so that its performance advantages can be extended to other public-health applications.

PI: Carraway, Jeff, Ph.D.

Determination of the Incidence and Pathology of Ventricular Enlargement in the Brains of NCTR's Sprague-Dawley (SD) Rats (E0743201)

Responsible Office: Office of Scientific Coordination
Collaborating Division: Neurotoxicology
Objective(s):

3) Determine the incidence of ventricular enlargement in the brains of NCTR's SD rat breeding colony through magnetic

resonance imaging (MRI).

4) Determine the presence and nature of any gross pathologic and/or histopathologic lesions/abnormalities in the brain and other tissues of the NCTR SD rat.
5) Determine the presence and role of any pathogenic microorganisms in rats with MRI-confirmed ventricular enlargement.
6) Determine if a correlation exists between the cases of acute death/limb paresis and ventricular enlargement.

PI: Chelonis, John J., Ph.D

Data Repository for Behavioral and Questionnaire Data (S00762)

Responsible Division: Neurotoxicology
Objective(s):

1) Develop a data repository for behavioral and questionnaire data collected in human-subject studies.
2) Allow for hypothesis testing with increased sample sizes.
3) Test additional hypothesis that were not proposed in the original studies in which data collection occurred.

PI: Chelonis, John J., Ph.D

Off-Site Clinical Collaborations Involving the NCTR Operant Test Battery (S00786)

Responsible Division: Neurotoxicology
Objective(s):
Develop and maintain collaborative research with outside laboratories that are using the NCTR Operant Test Battery. This research will provide validation data for the use of this instrument in human subjects as well as apply this test battery to topics of relevance to the FDA.

PI: Chelonis, John J., Ph.D.

System Test of NCTR's Multispecies Behavioral Test System (MBS) Upgrade: Development of a Fixed Consecutive Number (FCN) Task (E0751701)

Responsible Division: Neurotoxicology
Collaborating Office: Office of Scientific Coordination
Objective(s):
Provide a necessary step in the development and implementation of new MBS software to support administration of the FCN task which will provide new information about brain function that cannot be derived from the current Operant Test Battery tasks.

PI: Chen, James J., Ph.D.

Data-Mining Strategy to Identify Hepatotoxic Drugs and Sensitive Patients (E0740301)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):
Build a prototype computerized visualization system to integrate FDA safety data-mining system for further analysis of the FDA's Adverse Event Reporting System data. The system will allow similarities and differences among drugs and among events to be explored for further investigation.

PI: Chen, Tao, Ph.D.

Development and Evaluation of Exposure Dosimetry Methods to Optimize the Standard *In vitro* Mammalian Genotoxicity Assays for Assessing Engineered Nanomaterials (ENMs) (E0745701)

Responsible Division: Genetic and

Molecular Toxicology
Collaborating Divisions/Office: Neurotoxicology, Systems Biology, Office of Scientific Coordination
Objective(s):

1) Evaluate whether the *in vitro* mammalian genotoxicity assay is suitable for assessing the genotoxicity of nanomaterials.
2) Explore the possible mechanisms underlying genotoxicity of ENMs by conducting genomic analysis.
3) Identify potential improvements to the assay and general strategies for evaluating nanomaterials.
4) Examine whether the suitable methods and other experiences learned from the micronucleus assay are applicable to other genotoxicity tests, such as mouse lymphoma assay and *in vivo* micronucleus assay.

PI: Chen, Tao, Ph.D.

Do Engineered Silver Nanomaterials (Ag-ENMs) Varying by Size and Coatings Behave Differently Than Bulk Silver in Their Ability To Induce Genetic Damage? (E0750101)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Office: Office of Scientific Coordination
Objective(s):

1) Evaluate the Ames test and mouse lymphoma assay, in addition to the *in vitro* micronucleus assay.
2) Investigate Ag-ENMs of various sizes and compare to bulk silver results.

PI: Chen, Tao, Ph.D.

Evaluation of MicroRNAs (miRNAs) in Blood and Urine for Detection of Chemical-Induced Carcinogenicity (E0753001)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination
Objective(s):

1) Determine miRNAs in blood and carcinogenic target tissues that respond to exposure of carcinogens, and the best time for sampling of their expression after treatments in rats.
2) Determine miRNA profiles from the blood and target tissue samples of rats treated with different mode-of-action carcinogens, such as alkylating agents, aneugens, clastogens, and non-genotoxic carcinogens at the appropriate sampling time determined by Objective 1.
3) Determine the functions and pathways of the dysregulated miRNAs by the carcinogen treatments and examine whether the miRNA changes can be anchored to the carcinogens with the known mode-of-actions and whether the changes in blood relate to those in the target tissues.
4) Establish specific miRNA biomarkers in blood for assessing different types of carcinogens.

PI: Chen, Tao, Ph.D.

Evaluation of the Applicability of *In vivo* Micronucleus Assays for Assessing Genotoxicity of Engineered Nanomaterials (E0731001)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Divisions/Office: Biochemical Toxicology, Microbiology, Office of Scientific Coordination
Collaborating FDA Center: CFSAN
Objective(s):

1) Assess the genotoxicity of four types of nanoscale materials, carbon nanotubes, nanoscale titanium dioxide, nanoscale gold, and nanoscale silver in three standard tests suggested by FDA; *Salmonella* Ames test, mouse lymphoma assay, and *in vivo* mouse micronucleus assay.
2) Evaluate the possible mechanisms of nanomaterial-induced genotoxicity using a transgenic mutation system comet assay, and genomic analysis.

PI: Desai, Varsha G., Ph.D.

Development and Application of a Mitochondria-Specific Gene Array (Mitochip) for the Investigation of Clinical and Non-Clinical Predictive Biomarkers of Toxicity (E0739701)

Responsible Division: Systems Biology
Collaborating Divisions: Bioinformatics and Biostatistics, Neurotoxicology
Collaborating FDA Centers: CDER, CDRH
Objective(s):

1) Develop MitoChip for various mammalian species, including rat, nonhuman primate, and human.
2) Conduct transcriptional profiling of mitochondria-related genes using mitochondria-specific gene arrays to investigate the mechanisms of drug toxicities and degenerative diseases associated with mitochondrial dysfunction.
3) Characterize species-specific transcriptional profiles to predict risk of drug toxicity or disease-onset in different mammalian species.

PI: Desai, Varsha G., Ph.D.

Development of Predictive Mitochondrial Biomarkers for Drug-Induced Cardiotoxicity Using a Systems Biology Approach (E0733201)

Responsible Division: Systems Biology
Collaborating Divisions/Office: Biochemical Toxicology, Bioinformatics and Biostatistics, Office of Scientific Coordination
Collaborating FDA Center: CDER
Objective(s):

1) Measure heart rate and variability, using ECGenie.
2) Measure cardiac troponin T, creatine kinase MB, and cardiolipin levels in plasma as indicators of doxorubicin-induced cardiac-tissue damage.
3) Identify morphological changes in cardiac mitochondria in left ventricular region by electron microscopy.
4) Use omics for heart-analyte profiling: transcriptional profiling of ~906 mitochondria-related genes using MitoChip; protein profiling by 2D-HPLC/MS/MS, and measurement of endogenous metabolites by nuclear magnetic resonance and mass spectrometry.
5) Measure expression levels of 906 mitochondria-related genes in whole blood using MitoChip.
6) Measure levels of creatinine, creatine, lactate, Krebs cycle intermediates, small ketone bodies in plasma using metabolomics.
7) Integrate genomic, proteomic, and metabolomic endpoints in the heart tissue to define the molecular basis of doxorubicin-induced cardiac toxicity and also correlate omics data to genomic findings obtained in whole blood.

PI: Ding, Wei, Ph.D.

Development of 3-D Human Skin Model for *In vitro* Genotoxicity Testing of Chemical and Physical Agents (E0740001)

Responsible Division: Genetic and Molecular Toxicology
Collaborating FDA Center: CFSAN
Objective(s):

1) Evaluate the ability of 3-D human skin models to assess the genotoxicity of dermal exposure to FDA-regulated products, including nanomaterials.
2) Perform the Comet and MN assays with the 3-D human skin model to determine how each endpoint can be used to evaluate the genotoxicity of chemical and physical agents.

PI: Ding, Wei, Ph.D.

Validation of the *In vivo* Comet Assay for Pre-Market Submissions and Preparation of Detailed Review Paper To Assist in the Development of a New Organisation Economic Cooperation and Development Guideline (E0750401)

Responsible Division: Genetic and Molecular Toxicology
Collaborating FDA Center: CFSAN
Objective(s):

1) Review existing literature and identify critical information gaps.
2) Conduct limited research at NCTR.
3) Combine all information to prepare a detailed literature review that will serve as a background document for the new Organisation Economic Cooperation and Development guideline for the Comet assay.

PI: Dobrovolsky, Vasily N., Ph.D.

Development of a High-Throughput Assay for Measuring *In vivo* Mutation in an Autosomal Gene (E0741301)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Bioinformatics and Biostatistics
Objective(s):

1) Develop a high-throughput *in vivo* mutation model that detects mutations induced by a range of mechanisms, including gene mutation, large deletions, and loss of heterozygosity.
2) Evaluate the basic properties and sensitivity of the model in experiments employing well-characterized mutagens.

PI: Doerge, Daniel R., Ph.D.

Effect of Soy-Containing Diets on Ammonium Perchlorate-Induced Thyroid Toxicity in Sprague-Dawley Rats - II (Repeat Study) (E0742201)

Responsible Division: Biochemical Toxicology
Collaborating Division: Bioinformatics and Biostatistics
Objective(s):

Determine the effect of dietary whole soy and purified genistein, the principal soy isoflavone, on the dose-response characteristics for perchlorate-induced thyroid toxicity in male Sprague-Dawley rats.

PI: Fu, Peter P., Ph.D.

Computational Toxicology for Safety and Risk Assessment (S00780)

Responsible Division: Biochemical Toxicology
Objective(s):

The objective of this support request is

to assist other Centers in FDA (e.g., CFSAN and CDRH) and research organizations outside FDA (e.g., P & G and U.S. EPA).

PI: Fu, Peter P., Ph.D.

Determination of Cytotoxicity and Genotoxicity of Nanomaterials of Interest to the FDA and Mechanism of Action (E0752701)

Responsible Division: Biochemical Toxicology

Objective(s):

1) In conjunction with the erythrocyte sedimentation rate (ESR or SED rate) measurements made by Dr. Yin at CFSAN, to develop a set of cell-free and cell-based *in vitro* tests that can be used to rapidly identify nanomaterials of interest to the FDA that elicit oxidative damage.
2) Determine if, in the presence of nano-metal materials, endogenous and dietary antioxidants can display pro-oxidative activity.

PI: Fu, Peter P., Ph.D.

Mechanism of Tumorigenic Pyrrolizidine Alkaloids and Development of LC/ES/MS/MS Methodology for Detection and Quantification of Pyrrolizidine Alkaloids (E0728901)

Responsible Division: Biochemical Toxicology

Collaborating Division: Microbiology

Objective(s):

1) Validate the proposed mechanism by which pyrrolizidine alkaloids induce tumors in rodents.
2) Develop an LC/ES/MS/MS method for detection and quantification of DHP-derived DNA adducts in rodents.
3) Develop an LC/ES/MS/MS method for detection and quantification of genotoxic pyrrolizidine alkaloids in herbal plants and herbal dietary supplements.
4) Develop an LC/ES/MS/MS method for detection and quantification of DHP-derived hemoglobin adducts in rodents.

PI: Gu, Qiang, Ph.D.

Identification of Protein Biomarkers for Neurotoxicity Assessments Using a High-Throughput Antibody Microarray Approach (E0747701)

Responsible Division: Neurotoxicology

Objective(s):

1) Examine proteomic changes at both the expression and phosphorylation levels using five established *in vivo* models of neurotoxicity.
2) Identify common changes in protein expression and phosphorylation status in these animal-model systems.
3) Confirm the observed alterations in protein expression and phosphorylation status by means of other independent methods.
4) Apply the proteomic findings to a global ischemic animal model to further validate the utility of protein biomarkers for use in neurotoxicity assessments.

PI: Gu, Qiang, Ph.D.

Development of a Simple *In vitro* Approach for the Rapid Detection of Neurotoxicity (E0752401)

Responsible Division: Neurotoxicology

Collaborating FDA Center: CDER

1) Characterize Fluoro-Jade C (FJ-C) labeling *in vitro*. Experiments will be conducted in a variety of cell cultures to identify the types of cultured cells that can be labeled using FJ-C: neurons, astrocytes, oligodendrocytes, microglia, brain-capillary endothelial cells, or other non-neuronal cells. In addition, optimal

concentrations and incubation durations will be determined for FJ-C use *in vitro* and we will determine whether FJ-C itself can be neurotoxic in culture media.

2) Validate FJ-C labeling *in vitro*. Experiments will be performed using several well-known neurotoxicants such as tetrodotoxin, lead, mercury, cadmium, ethanol, biphenyl compounds and others to confirm FJ-C labeling for neurodegeneration *in vitro* and to determine dose-dependent and time-dependent effects of these toxic compounds on FJ-C labeling as reference parameters of neurotoxicity *in vitro*.
3) Develop an FJ-C-based *in vitro* approach for high-throughput determination of neurotoxicity. Experiments will be designed to combine FJ-C labeling, multi-well culture plates, and high-content time-lapsed recordings to achieve the goal of simple, fast, multiplexed, efficient, and accurate screens and analyses of neurotoxic compounds.
4) Explore the mechanism underlying FJ-C labeling. Studies will be undertaken in attempts to identify the 'death' molecule(s) that bind FJ-C.

PI: Gu, Qiang, Ph.D.

Identification of Protein Biomarkers for Neurotoxicity Assessments Using a High-Throughput Antibody Microarray Approach (E0747701)

Responsible Division: Neurotoxicology
Collaborating Division: Systems Biology
Objective(s):

1) Examine proteomic changes at both the expression and phosphorylation levels using five established *in vivo* models of neurotoxicity.
2) Identify common changes in protein expression and phosphorylation status in these animal model systems.
3) Confirm the observed alterations in protein expression and phosphorylation status by means of other independent methods.
4) Apply the proteomic findings to a global ischemic animal model to further validate the utility of protein biomarkers for use in neurotoxicity assessments.

PI: Hanig, Joseph P., Ph.D.

ADDENDUM to E0741801: Development of Magnetic Resonance Imaging (MRI) and Informatics Techniques for Tissue Sampling to Guide and Confirm Classical Neuropathology (E0741811)

Responsible Division: Neurotoxicology
Collaborating Center: CDER
Objective(s):

1) Build dose-response and time-course curves of trimetyltin and hexachlorophene neurotoxicity using MRI T2 mapping.
2) Assess sensitivity and specificity of T2 mapping in relation to histopathology using Receiver Operating Characteristic curves approach.
3) Assess neurotoxicological effect of mefloquine.

PI: Hanig, Joseph P., Ph.D.

Development of Magnetic Resonance Imaging (MRI) and Informatics Techniques for Tissue Sampling to Guide and Confirm Classical Neuropathology (E0741801)

Responsible Division: Neurotoxicology
Collaborating FDA Center: CDER
Objective(s):

Use MRI and informatic analysis of MRI files to screen brain samples for neuro-irregularities (presumed toxicities) that

would inform and direct the plane and loci of slices or sections taken for confirmatory classical neuropathology.

PI: Hansen, Deborah K., Ph.D.

Development of the Mouse Embryonic Stem-Cell Test (E0741901)

Responsible Division: Systems Biology
Collaborating Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):
Gain hands-on experience with this test to better characterize it and indicate potential modifications to the assay.

PI: He, Zhen, Ph.D.

Brain Sexual Dimorphic Structures and Sex Hormone-Like Compounds (SHLC) (P00710)

Responsible Division: Neurotoxicology
Objective(s):
Establish a series of standardized procedures for evaluating SHLC-induced changes in brain morphology utilizing immmunohistochemical and other, more traditional techniques.

PI: Heflich, Robert H., Ph.D.

Phosphatidylinositol Glycan Complementation Group A (Pig-a) Mutagenesis: An International Validation Study Comparing Pig-a Mutation in Rats with Other Biomarkers of Genetic Toxicity (E0741201)

Responsible Division: Genetic and Molecular Toxicology
Collaborating FDA Centers: CDER, CVM
Objective(s):

1) Generate data using a standardized protocol that, in combination with results from other investigators, will be used to determine the sensitivity, specificity, and portability of the rat red blood cell (RBC)/reticulocytes (RET) Pig-a gene mutation assay.
2) Determine how the RBC/RET Pig-a assay compares in terms of sensitivity and specificity with these other *in vivo* assays that have been used or considered for use as regulatory assays by performing the *in vivo* Comet, micronucleus, and the Pig-a and Hprt lymphocyte gene mutation assays in conjunction with the RBC/RET Pig-a assay.

PI: Hong, Huixiao, Ph.D.

Further Development and Refinement of the FDA Endocrine Disruptor (ED) Knowledge Base (EDKB) for Assessing Endocrine Disrupting Potential of Drugs and Food Additives (E0741501)

Responsible Division: Bioinformatics and Biostatistics
Collaborating Divisions/Office: Biochemical Toxicology, Systems Biology, Office of Scientific Coordination
Objective(s):

1) Improve EDKB by including the ED data that will be generated by NTP and NCTR as well as estrogen receptor and androgen receptor data generated at EPA, and data that have been published in the past eight years.
2) Conduct meta-analyses of the large datasets accumulated in the EDKB to gain a better understanding of chemical structure requirement and mechanisms related to EDs.
3) Investigate various chemoinformatics/ bioinformatics approaches to develop effective predictive models for assessing the potential of drugs and food additives.

PI: Howard, Paul C., Ph.D.

Analytical Assay for Photochemical Generation of Hydroxyl Radical (S00728)

Responsible Office: Office of Scientific Coordination
Collaborating Division: Biochemical Toxicology
Objective(s):

1) Provide support for analysis of the photoactivation of nanomaterials using the OH/coumarin-3-carboxylic acid assay.
2) Provide particle-size analysis for all materials being analyzed by OH method and other nanomaterials used in studies at FDA's NCTR and ORA/Arkansas Regional Laboratory.
3) Improve the assay using ultraviolet light diode laser as a replacement to the existing broad-band ultraviolet light-A source.

PI: Howard, Paul C., Ph.D.

NCTR/Office of Regulatory Affairs (ORA) Nanotechnology Core Facility—FDA SUPPORT (S00714)

Responsible Division: Office of Scientific Coordination
Collaborating FDA Office: ORA
Objective(s):

1) Support the needs of NCTR to characterize nanoscale materials used in toxicology tests and to detect these materials in biological samples.
2) Support the needs of FDA's ORA/Arkansas Regional Laboratory to detect and characterize nanoscale materials in FDA-regulated products.

PI: Howard, Paul C., Ph.D.

NCTR/Office of Regulatory Affairs (ORA) Nanotechnology Core Facility—NTP IAG SUPPORT (S00715)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Office: ORA
External Partner: National Toxicology Program
Objective(s):

1) Support the needs of NCTR to characterize nanoscale materials used in toxicology tests and to detect these materials in biological samples.
2) Support the needs of ORA/Arkansas Regional Laboratory to detect and characterize nanoscale materials in FDA-regulated products.

PI: Howard, Paul C., Ph.D.

Support of Collaborative Projects in Nanotechnology with Baylor University and University of Texas Health Science Center (S00774)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Office: ORA
External Partners: Baylor University, University of Texas Health Science Center
Objective(s):
Provide analytical support for small or investigative projects in collaboration with investigators at Baylor University and University of Texas Health Science Center.

PI: Howard, Paul C., Ph.D.

Support of Collaborative Projects in Nanotechnology with FDA/Center for Drug Evaluation and Research (CDER) (S00770)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Center/Office: CDER, ORA
Objective(s):

Provide analytical support for small or investigative projects in collaboration with investigators at FDA/CDER.

PI: Howard, Paul C., Ph.D.

Support of Collaborative Projects in Nanotechnology with FDA/Center for Devices and Radiological Health (CDRH) (S00769)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Center/Office: CDRH, ORA
Objective(s):
Provide analytical support for small or investigative projects in collaboration with investigators at FDA/CDRH.

PI: Howard, Paul C., Ph.D.

Support of Collaborative Projects in Nanotechnology with FDA/Center for Food Safety and Nutrition (CFSAN) (S00771)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Center/Office: CFSAN, ORA
Objective(s):
Provide analytical support for small or investigative projects in collaboration with investigators at FDA/CFSAN.

PI: Howard, Paul C., Ph.D.

Support of Collaborative Projects in Nanotechnology with FDA/Center for Veterinary Medicine (CVM) (S00772)

Responsible Division: Office of Scientific Coordination
Collaborating FDA Center/Office: CVM, ORA
Objective(s):
Provide analytical support for small or investigative projects in collaboration with investigators at FDA/CVM.

PI: Howard, Paul C., Ph.D.

Support of Collaborative Projects in Nanotechnology with FDA/Office of Regulatory Affairs (ORA) (S00773)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Office: ORA
Objective(s):
Provide analytical support for small or investigative projects in collaboration with investigators at FDA/ORA.

PI: Howard, Paul C., Ph.D.

Support of Collaborative Projects in Nanotechnology with ISLI/HESI (S00783)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Office: ORA
Objective(s):
Provide analytical support for small or investigative projects in collaboration with investigators at FDA/ORA.

PI: Howard, Paul C., Ph.D.

Support of Collaborative Projects in Nanotechnology with University of Arkansas at Fayetteville (S00767)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Office: ORA
External Partner: University of Arkansas at Fayetteville
Objective(s):
Provide analytical support for small or investigative projects in collaboration with investigators at University of Arkansas at Fayetteville.

PI: Howard, Paul C., Ph.D.

Support of Collaborative Projects in Nanotechnology with University of Arkansas at Little Rock (S00766)

Responsible Office: Office of Scientific Coordination

Collaborating FDA Office: ORA
External Partner: University of Arkansas at Little Rock
Objective(s):
Provide analytical support for small or investigative projects in collaboration with investigators at University of Arkansas at Little Rock.

PI: Howard, Paul C., Ph.D.

Support of Collaborative Projects in Nanotechnology with University of Arkansas for Medical Sciences (S00768)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Office: ORA
External Partner: University of Arkansas for Medical Sciences
Objective(s):
Provide analytical support for small or investigative projects in collaboration with investigators at University of Arkansas for Medical Sciences.

PI: Inselman, Amy L., Ph.D.

Establishment of Embryonic Stem (ES) Cells as an *In vitro* Model to Explore Developmental Toxicity (E0735401)

Responsible Division: Systems Biology
Collaborating Division: Bioinformatics and Biostatistics
Objective(s):
1) Maintain and passage several mouse ES and human-induced pluripotent stem (iPS) cell lines in a pluripotent, undifferentiated state in the absence of feeder cells and serum.
2) Recapitulate early embryonic development by terminal differentiation of mouse ES and human iPS cells into a variety of cell types (i.e. osteoblasts).
3) Monitor this differentiation process by examining gene expression in undifferentiated ES and in cells that have undergone differentiation.
4) Provide proof-of-concept by using known teratogens and investigating gene changes in differentiated cells, such as acetazolamide treatment of differentiating osteoblasts.

PI: Khan, Ashraf A., Ph.D.

Detection of Cytolethal Distending toxin (cdtB), pltA and pltB Homologs of Components of the Pertussis Toxin Genes by Polymerase Chain Reaction (PCR) and Studies on Functionality of cdtB in Non-Typhoidal *Salmonella* spp. (E0739601)

Responsible Division: Microbiology
Collaborating Office: Office of Scientific Coordination
Collaborating FDA Center/Office: CFSAN, ORA
Objective(s):
1) Develop a duplex real-time, quantitative polymerase chain reaction (qPCR) to detect *Salmonella* and antibiotic-resistance gene markers simultaneously from food samples.
2) Validation of qPCR method at FDA, ORA laboratories.
3) Characterize plasmids, virulence genes, and integrons, for their role in virulence in multidrug-resistant *Salmonella* strains isolates from food.

PI: Kanungo, Jyotshnabala, Ph.D.

ADDENDUM to E0735901: Methods Development for Toxicity Assays using the Zebrafish Embryo as a Model System: Whole Animal High-Throughput Assays for Chemical Testing (E0735911)

Responsible Division: Neurotoxicology
Collaborating Office: Office of Scientific Coordination
Objective(s):

1) Study the effect of METH on zebrafish embryos, especially relating to sensory and motor-neuron development.
2) Determine if carbon nanotubes pass through the blood-brain barrier in zebrafish embryos and have any toxic effects on early development. If so, we will determine whether these nanomaterials generate reactive oxygen species, cause the depletion of dopamine and its metabolites, dihydroxyphenylacetic acid and homovanillic acid, and alter markers of oxidative stress.
3) Study the effect of nicotine on zebrafish embryos, especially relating to sensory and motor neuron development and the mechanism of action.

PI: Kanungo, Jyotshnabala, Ph.D.

Methods Development for Toxicity Assays Using the Zebrafish Embryo as a Model System: Whole Animal High-Throughput Assays for Chemical Testing (E0735901)

Responsible Division: Neurotoxicology
Collaborating Office: Office of Scientific Coordination
Objective(s):
Establish a high-throughput assay system using zebrafish embryos to monitor both traditional morphological and behavioral endpoints of toxicity and the newer, more subtle organ-specific toxicities of FDA-relevant compounds.

PI: Khan, Ashraf A., Ph.D.

Screening of Dietary Supplements for *Bacillus* Contamination Using Chromogenic Agar: Identification of Enterotoxigenic *Bacillius Cereus* Group and Pre-Formed Emetic Toxin (E0748301)

Responsible Division: Microbiology
Collaboborating Office: Office of Scientific Coordination
Collaborating FDA Center: CFSAN
Objective(s):
Improve the detection of enterotoxigenic *B. cereus* from dietary supplements and food products. Confirmation of the bacteria from incriminated dietary supplements and foods is complicated by the presence of background microorganisms. *B. cereus* is not competitive with other organisms; therefore, culture media with additional agents to suppress background microorganisms will be evaluated.

PI: Khan, Saeed A., Ph.D.

Does the Durable Nanoparticle Bioaccumulation in Macrophages Increase Susceptibility to Bacterial Infection? (E0753601)

Responsible Division: Microbiology
Collaborating Division/Office: Systems Biology, Office of Scientific Coordination
Collaborating FDA Center: CDER
Objective(s):
Determine whether animals exposed to durable nanoparticles are more susceptible to *Listeria* infection as measured by the severity of disease and the length of time needed to clear the infection.

PI: Khare, Sangeeta, Ph.D.

Interaction of Nanoparticles with Gastrointestinal Tract (E0744301)

Responsible Division: Microbiology
Collaborating Office: Office of Scientific Coordination
Objective(s):
1) Determine the effect of nanomaterials on the permeability of epithelial cells and establish immune correlates.

2) Delineate the interaction of nanomaterials with gastro-intestinal tract and gut-associated microbiota using *ex vivo* model (intestinal explants).
3) Establish the effect of nanoparticle on the developmental stage of intestine and assess biodistribution of nanoparticle using zebrafish model.

PI: Kim, Sung, Ph.D.

Microbiological Diagnostic Methods: Development, Testing, & Evaluation (E0026200)

Responsible Office: Office of Scientific Coordination

Objective(s):

Improve diagnostic and epidemiological capabilities in bacteriology, parasitology, mycology, virology, and serology as applicable to NCTR programs and projects.

PI: Leakey, Julian E., Ph.D.

Complement Assays for the Detection of Immumo-Sensitizing Activity of Nanomaterials (E0754501)

Responsible Office: Office of Scientific Coordination

Objective(s):

1) Establish two complement assays at NCTR for routine evaluation of immuno-sensitizing activity of nanomaterials.
2) Validate the assays using nanoparticles with known immunoreactivity and determine the immuno-sensitizing activity of novel nanomaterials.

PI: Liachenko, Serguei, Ph .D.

The Use of Magnetic Resonance Imaging (MRI) in the Quantification of Stroke and its Consequences (E0739901)

Responsible Office: Office of the Director

External Parner: University of Arkansas for Medical Sciences

Objective(s):

1) Demonstrate the ability to detect and follow microbleeds using NCTR's state-of-the-art 7 tesla MRI.
2) Characterize with MRI the anatomy of those microbleeds that may go on to symptomatic or asymptomatic hemorrhage.
3) Relate MRI findings in the rabbit to neurological and anatomical changes, such as those involved in dementia.
4) See if standard tPA therapy or one of the novel therapies involved in the treatment of acute stroke affect microbleed qualities.

PI: Liu, Zhichao, Ph.D.

Drug Repositioning with Bioinformatics (E0747001)

Responsible Division: Bioinformatics and Biostatistics

Collaborating Division/Office: Systems Biology, Office of Scientific Coordination

Objective(s):

Use bioinformatics to explore repositioning opportunities of marketed drugs (prescription and over-the-counter) for various diseases (including rare and neglected diseases).

PI: Manjanatha, Mugimane, Ph.D.

Development of Methods for Evaluating DNA Damage Using Single-Cell Gel Electrophoresis (Comet Assay) in Rodents (E0729001)

Responsible Division: Genetic and Molecular Toxicology

Collaborating FDA Center: CFSAN

Objective(s):

Evaluate and establish methods and conditions that enhance the sensitivity and reproducibility of the *in vivo* alkaline-comet assay for use in

preclinical-hazard identification and genotoxicity testing of food ingredients and chemicals for regulatory purposes.

PI: Manjanatha, Mugimane, Ph.D.

Validation of a Newly Developed Transgenic, Hairless, and Albino Mice (E0727701)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination
Objective(s):

1) Analyze tissue-specific mutant frequency and spectra using transgenic systems to evaluate a variety of hypotheses on the mechanisms or mode-of-action for cancer induction in rodents.
2) Facilitate improvements in human-risk characterization based on extrapolation from animal data.

PI: Mattes, William B., Ph.D.

Biomarkers of Liver Toxicity (E0732201)

Responsible Division: Systems Biology
Collaborating Division: Bioinformatics and Biostatistics
Objective(s):

1) Discover biomarkers of hepatotoxicity in preclinical studies that are more predictive of adverse effects in humans. These biomarkers may or may not be directly applicable to the clinic, but they will be predictive of human responses so that they can be used to extrapolate preclinical data to humans in safety assessments.
2) Qualify these biomarkers (e.g., via the FDA/EMEA qualification process) and potential translation for clinical use.

PI: McKinzie, Page, Ph.D.

Development of Cancer-Relevant Biomarkers for Identification of Potential Carcinogens: Research To Understand the Normal Background Frequencies in Rats (E0733601)

Responsible Division: Genetic and Molecular Toxicology
Objective(s):
Understand the distribution and range of spontaneous oncogene-mutant frequencies in the major organs of rats and mice to provide important basic information for the validation of these oncogene-mutant frequencies as biomarkers of chemically induced carcinogenesis.

PI: Nakamura, Noriko, Ph.D.

Development of an Immunohistochemical Tool To Measure Degree and Distribution of Global Epigenetic Alterations in Liver Tissue Samples (E0755201)

Responsible Division: Systems Biology
External Partners: Central Arkansas Veterans Healthcare Systems, Duke University, University of Arkansas for Medical Sciences
Objective(s):

1) Develop an immunohistochemical tool to assess degrees of global DNA methylation and histone protein acetylation in the liver along with their distributions.
2) Develop an immunohistochemical approach as a tool for evaluating the alteration of epigenetic modifications using various tissues section (including the liver) from mice treated with/without acetaminophen.

3) Determine the correlations between the degree of epigenetic alterations, gender, and severity of liver injury by statistical analyses.
4) Perform and optimize three immunohistochemical stains to investigate the degree and distribution of global DNA methylation and histone acetylation in the liver in a quantifiable manner.
5) Develop a histologic scoring system to systematically assess these global epigenetic alterations for ease of comparison with future studies using human liver tissue samples.
6) Correlate the quantitative data (on DNA methylation, histone acetylation) with the severity of acetaminophen-induced liver injury.

PI: Paredes, Angel, Ph.D.

Collaboration on Nanotechnology and Electron Microscopy with St. Jude Children's Research Hospital (S00779)

Responsible Office: Office of Scientific Coordination
External Partner: St. Jude Research Hospital
Objective(s):
The objective is for the NCTR/ORA Nanotechnology Core Facility to provide analytical and electron microscopy support form small investigative projects in collaboration with investigators at St. Jude Children's Research Hospital.

PI: Perkins, Roger

CTP Bioinformatics for Text and Topic Modeling (E0755801)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CTP
Objective(s):
Create a tool to structure large document collections obtained from tobacco companies in response to CTP regulatory actions. Evaluating these document collections without such a tool presents a daunting, expensive and protracted task. The tool provides a means to:

a. Make queries on a particular regulatory questions, resulting in probability-ranked lists of which documents are most likely to yield the desired information.
b. Vet document collections, culling the uninformative ones, while retaining and annotating the most informative with metadata (e.g., health-related, marketing, demographic targeting, additives etc.)
c. Perform linguistic investigations into language, semantic context, and semantic jargon used by tobacco companies to inform CTP development of lexicons, controlled vocabularies and/or ontologies.

PI: Perkins, Roger G.

CTP Bioinformatics Tobacco Constituents Knowledge Base (TCKB) and of Harmful and Potentially Harmful Constituents (HPHC) Toxicology (E0755901)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CTP
Objective(s):
The TCKB will be a versatile and authoritative repository for chemical-centric information, including chemical structure, and associated biological toxicity and endpoint information; data can be accumulated from all sources,

and linked from the database to source references.

PI: Petibone, Dayton, Ph.D.

Differential Transcriptomic Characterization of TK6 and WTK1 Human Lymphoblast Cells by Next-Generation RNA Sequencing (E0744001)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Divisions: Bioinformatics and Biostatistics, Systems Biology
Objective(s):

1) Develop next-generation pyrosequencing capabilities for RNA-sequence analysis and genomic sequencing in order to characterize the TK6 and WTK1 transcriptosome.
2) Apply technology to the determination of the baseline whole-genome gene expression levels in each cell line.
3) Measure transcriptosome response in TK6 and WTK1 after exposure to classical positive control agents, which may include the direct-acting alkylating agent, ENU, as well as ionizing radiation. Other positive control agents may be selected during the conduct of the protocol.

PI: Schnackenberg, Laura, Ph.D.

Using Cell-Free MicroRNA (miRNA) as Improved Clinical Biomarkers of Drug-Induced Liver Injury (DILI) Method Development of MALDI Imaging Mass Spectrometry (P00782)

Responsible Division: Systems Biology
Collaborating Division: Neurotoxicology
Objective(s):

1) Develop methods for analysis of rat-brain tissue slices including tissue preparation, matrix deposition, and data acquisition.
2) Analyze rat-brain tissue slices after exposure to iron nanoparticles. The method development of Obective #1 will be employed for evaluation of nanoparticle distribution in rat-brain slices after exposure to iron nanoparticles. Comparisons will be made with associated MRI data.
3) Analyze other tissues or whole-body sections.

PI: Shi, Qiang, Ph.D.

Preclinical Studies Investigating the Dose Range and Proof-of-Principle that Leflunomide-Induced Liver Injury (LILI) is Enhanced by Cytochrome P450 Inhibition (E0744201)

Responsible Division: Systems Biology
Collaborating Office: Office of Scientific Coordination
Objective(s):

1) Develop a rat model of LILI focusing on the involvement of hepatic cytochrome P450s.
2) Determine if a preclinical model of LILI can be established to help in the development of novel biomarker(s) to assess individual sucsceptibility to LILI in future studies.

PI: Shi, Qiang, Ph.D.

Using Cell-Free MicroRNA (miRNA) as Improved Clinical Biomarkers of Drug-Induced Liver Injury (DILI) (E0749701)

Responsible Division: Systems Biology
Collaborating Division: Bioinformatics and Biostatistics
Collaborating FDA Center/Office: CDER, Office of Chief Scientist
Objective(s):

1) Measure the level of miRNAs in human serum, urine, and liver samples from patients experiencing DILI and normal healthy controls.

2) Compare the changes in miRNA levels between groups to identify specific miRNAs that may serve as new biomarkers of DILI.

PI: Slavov, Svetoslav, Ph.D.

Development and Validation of 3D-QSDAR Models for Prediction of the Binding Affinity of Chemicals from the ToxCast Database to the Estrogen Receptor (ER) (E0753901)

Responsible Division: Systems Biology
External Partner: U.S. Environmental Protection Agency
Objective(s):

1) Build and validate 3D-QSDAR models for ER binding.
2) Search for structural patterns in the data.
3) Decode the structure-activity relationship.
4) Provide validated models for a reliable estimation of the ER binding affinity of a diverse dataset of chemicals.
5) Identify the structural features responsible for binding to ER.
6) Provide a list of chemicals prioritized for further laboratory testing.
7) Send model and structural features to EPA for combination with other models being provided by collaborators.

PI: Sutherland, John B., Ph.D.

Reducing Health Risks from Antimicrobial-Resistant Bacteria by Eliminating Environmental Reservoirs of Resistance (E0738201)

Responsible Division: Microbiology
Collaborating Divisions/Office: Biochemical Toxicology, Systems Biology, Office of Research
Collaborating FDA Center: CVM
Objective(s):
Identify the specific bacteria and enzymes in the environment that are able to degrade fluoroquinolones to products without antimicrobial activity.

PI: Tolleson, William H., Ph.D.

Evaluating Conventional Methods for Thermal and Chemical Inactivation of the Bioterrorism Agent—Ricin—Contaminating Pilot-Scale Milk Pasteurization Equipment (E0746701)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CFSAN
Objective(s):

1) Determine the residual biological activity remaining for ricin-contaminated milk processed using the range of time/temperature conditions recommended for pasteurization of raw milk using pilot-scale (4L) equipment safely within the secure, high-level biosafety level 3 food-processing facility located at the National Center for Food Safety and Technology.
2) Determine the efficacies of chemical inactivation methods developed on a laboratory-scale to decontamination of food-processing equipment on a pilot-scale.

PI: Tong, Weida, Ph.D.

Development and Refinement of the FDA Genomic Tool, ArrayTrack™ for Advancing Pharmacogenomics and Personalized Medicine Supporting FDA's Critical Path Initiative (S00671)

Responsible Division: Bioinformatics and Biostatistics
Collaborating Division: Systems Biology
Collaborating FDA Center: CDER
Objective(s):

1) Analyze data from CDER drug review offices using ArrayTrack™ and return results to CDER collaborators.
2) Develop the functionality in ArrayTrack™ to review non-microarray PGx data, supporting the Critical Path Initiative.
3) Develop new modules in ArrayTrack™ to review proteomic, metabolomic, and genome-wide association studies data.
4) Develop modules to allow electronic data submission in the Voluntary Genomic Data Submission (VGDS)/Voluntary Exploratory Data Submission (VXDS) program.

PI: Tong, Weida, Ph.D.

FDA IT Support (S00699)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
External Partner: Clinical Data Interchange Standard Consortium (CDISC)
Objective(s):

1) Janus will integrate submitted review data from pre-clinical, clinical, and omics domains with external scientific data.
2) NCTR's ArrayTrack™software will be integrated with Janus to provide omics data capability. Janus will enable electronic data submission and review.

PI: Tong, Weida, Ph.D.

MicroArray Quality Control (MAQC) Project Database (S00691)

Responsible Division: Bioinformatics and Biostatistics
Collaborating Division: Systems Biology
Objective(s):

1) Update MAQC database when new data become available.
2) Maintain and regularly back up database at NCTR.

PI: Tong, Weida, Ph.D.

SEQC (MACQ-III) —The Sequencing Quality Control Project (E0731901)

Responsible Division: Bioinformatics and Biostatistics
Collaborating Division/Office: Systems Biology, Office of Scientific Coordination
Collaborating FDA Centers: CFSAN, CDER, CDRH
Objective(s):

1) Assess different next-generation sequencing (NGS) technologies and various bioinformatics strategies for handling and analyzing the massive sequence datasets by using the reference RNA samples previously established by the MicroArray Quality Control (MAQC) project.
2) Profile, using NGS technologies, RNA samples isolated from cells with or without treatment by nanoparticles and known toxicants to further evaluate their performance in assessing the safety and toxicity of FDA-regulated products.

PI: Varma, Vijayalakshmi, Ph.D.

An Omics Approach To Investigate the Metabolic and Endocrine Effects of Fructose on Adipocytes Compared to Glucose (E0740401)

Responsible Division: Systems Biology
Collaborating Division: Bioinformatics and Biostatistics
Collaborating FDA Center: CFSAN
External Partner: University of Arkansas for Medical Sciences
Objective(s):
Identify cellular mechanisms involved in fructose-induced metabolic and endocrine regulation of human

adipocytes in culture using omic technologies.

PI: Wang, Yuping, Ph.D.

Study of Translational Biomarkers for Drug-Induced Liver Injury (DILI) with Next-Generation Sequencing (NGS) (E0753201)

Responsible Division: Bioinformatics and Biostatistics
Collaborating Division: Genetic and Molecular Toxicology
Objective(s):
Conduct a comprehensive survey of miRNA using NGS technology. The resulting findings will elucidate the molecular pathways and processes modulated by RNAs (including mRNAs, miRNAs and other non-coding RNAs) and their importance in DILI risk and phenotypes. It is anticipated that miRNA biomarkers from rat may be more predictive for human-specific DILI than mRNA alone.

PI: Wilkes, Jon G., Ph.D.

Pilot Study: "Non-Invasive Magnetic Resonance Spectroscopy (MRS) Diagnosis of Diffuse Tissue Brain Diseases" (P00778)

Responsible Division: Systems Biology
External Partner: Huntington Medical Research Institute
Objective(s):

1) Obtain historical Alzheimer's and traumatic brain injury (TBI) patient MRS scans from Huntington Medical Research Institute (HMRI).
2) Process MRS data using NCTR developed novel pre-processing methodology to increase spectral accuracy.
3) Develop pattern-recognition models for each data set.
4) Produce accurate results for non-invasive diagnosis of Alzheimer's Disease and TBI using MRS scans.

PI: Yu, Li-Rong, Ph.D.

Metabolomics and Proteomics Approaches Addressing Pre-Analytical Variability in Human Plasma Samples (E0755601)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CDER
External Partner: Metanomics Health GmbH
Objective(s):
Establish a cooperative research and collaboration agreement (CRADA) between Metanomics Health GmbH and NCTR in the field of Quality Assurance and Quality Control that will discover biomarkers of metabolomics and proteomics sample quality related to variations in pre-analytical processing of clinical plasma samples. The metabolomics labs at the NCTR will become a tester of the proprietary metabolomics sample quality biomarkers developed at Metanomics Health.

NCTR Objective 1.3 – Approaches for Promoting Individualized Health and Identifying Susceptible Subpopulations

PI: Beger, Richard D., Ph.D.

Delta Vitamin Obesity Prevention Summer Camp (E0733001)

Responsible Division: Systems Biology
Collaborating Division: Microbiology
External Partners: U.S. Department of Agriculture/Agricultural Research Service, Marvell Community Development Center, University of Arkansas at Medical Sciences
Objective(s):

1) Analyze levels of 13 vitamins in 100 children in grades 4-6 to confirm food-frequency questionnaire data showing low intakes of certain nutrients and vitamins.
2) Provide fresh fruits, vegetables, and fortified snacks to supplement low-vitamin intake for a one-month period to improve serum concentration levels of vitamins.
3) Analyze ancestry through whole-genome scans and candidate genes responsive to vitamin intake to associate individual responses with genetic polymorphisms.
4) Improve the nutrition and genetic education of the participants through lessons taught by local teachers with materials provided by NCTR, U.S. Department of Agriculture, and the local University of Arkansas for Medical Sciences' Area Health Education Centers diabetes educator.
5) Develop health-economic analyses of the intervention.
6) Begin developing a sustainable program for improving the foods of the children in the Marvell School District by analyzing economic impact of vitamin intervention.

PI: Beger, Richard D., Ph.D.

Identification of New Mechanistic Biomarkers of Adverse Responses to Acetaminophen (APAP) (E0731301)

Responsible Division: Systems Biology
Objective(s):

1) Identify specific adduct proteins in children/adolescents receiving therapeutic doses of APAP (P-adducts) and in children/adolescents that have received APAP overdoses (T-adducts).
2) Examine metabolomic markers in these patients to address the role of redox status and energy metabolism in the study population.
3) Establish 2nd-generation biomarkers of APAP toxicity using the data generated from this study, based on specific adduct proteins, which can be used in future risk assessment studies of children receiving APAP.

PI: Chang, Ching-Wei, Ph.D.

Integrated Analysis of Single Nucleotide Polymorphism (SNP) and Copy Number Variation in Genome Association of Breast Cancer (E0744401)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Centers: CBER, CDRH
Collaborating FDA Office: Office of Women's Health
Objective(s):

1) Develop new statistical methods to integrate single locus changes (i.e., SNP) with DNA copy-number variation and assess association with breast-cancer status with these measures of genetic variability.
2) Identify genetic components of susceptibility to breast-cancer risk to allow early prevention and interventions to reduce suffering and loss of life due to breast cancer.

PI: Chang, Ching-Wei, Ph.D.

Blood Pressure (BP) Threshold for Cardiovascular Risk: An Assessment of Sex-Based Criterion (E0754201)

Responsible Division: Bioinformatics and Biostatistics
Collaborating Division: Systems Biology
Collaborating FDA Center: CDER
Objective(s):

1) Review systematically existing literature and develop a database for BP and Ambulatory Blood Pressure Monitoring (ABPM) measurements, hypertension, and cardiovascular risk.
2) Conduct meta-analyses of studies in the database developed in #1 above and perform sensitivity analysis to determine thresholds for assessing cardiovascular risk.
3) Determine whether BP or ABPM measurements from clinical studies warrant modifications for sex-based thresholds for cardiovascular risk and whether this criterion should differ for pre- and post-menopausal women.

PI: Chelonis, John J., Ph.D.

ASK CHILDREN Study—Assess Specific Kinds of Children Challenges for Neurologic Devices (E0734301)

Responsible Office: Neurotoxicology
Objective(s):

1) Establish a science-based framework of recommendations to help develop more efficient strategies in evaluating pediatric products regulated by FDA.
2) Develop a framework of science-based recommendations important to help expedite pediatric prostheses to market, including recommendations for the research and development of neurologic devices.
3) Collect qualitative and quantitative self-report clinical data (through interviews) and identify scientific and medical issues associated with pediatric devices when used in children undergoing treatment, to develop more efficient strategies for evaluating these types of products regulated by FDA.
4) Organize data that are important to developing more efficient strategies in evaluating these types of products regulated by FDA into multiple categories, including (but not limited to); device type, pediatric subpopulations, disorder or condition, and intended use.

PI: Chelonis, John J., Ph.D.

Complex Brain-Function Study in Children With and Without Major Depression (E0717701)

Responsible Division: Neurotoxicology
Objective(s):
Determine if children diagnosed with major depression according to the Diagnostic and Statistical of Mental Disorders criteria perform differently than children without such a diagnosis on tests of motivation, simple visual discrimination, timing ability, memory, and learning.

PI: Chelonis, John J., Ph.D.

Development and Validation of Interspecies Cognitive Assessments (E0735501)

Responsible Division: Neurotoxicology
Collaborating Office: Office of Research
Objective(s):
Compare children's performance on operant tests (that have been used extensively to assess drug effects in animals) with performance on neuropsychological tests (that are typically used in clinical settings that are thought to measure similar cognitive functions).

PI: Chelonis, John J., Ph.D.

Effects of Anxiety on Complex Brain Function in Children (E0721701)

Responsible Division: Neurotoxicology
Objective(s):
Determine if children with high levels of anxiety perform differently than children without anxiety on tests of motivation, simple visual discriminations, timing ability, memory, and learning.

PI: Chen, James J., Ph.D.

Predicting Patient-Specific Treatment Outcomes: Identification and Validation of Molecular Biomarkers Using *In silico* Tools (E0748601)

Responsible Division: Bioinformatics and Biostatistics
Collaborating FDA Centers: CBER, CDER, CDRH
Objective(s):
Develop statistical and data-mining techniques to identify personalized biomarkers to characterize individual differences in response to treatment and to understand disease progression: application to lung-cancer therapies.

PI: Chen, Minjun, Ph.D.

Biomarker Study To Improve Adjuvant Treatment for ER-positive and HER2-Negative Breast-Cancer Patients (E0748401)

Responsible Division: Bioinformatics and Biostatistics
Collaborating Office: Office of Scientific Coordination
Objective(s):
1) Assess the utility and effectiveness of the potential biomarker in predicting treatment oucomes of chemotherapy for breast-cancer patients with positive estrogen-receptor (ER) and negative human epidermal growth factor receptor-2 (HER2) based on breast-cancer tissue arrays using immunohistochemistry.
2) Study the role of the potential biomarker in breast-cancer development at both gene and protein levels using human breast-cancer cell lines.

PI: Desai, Varsha G., Ph.D.

Establishing a Mouse Model of Sex-Related Differences in Doxorubicin (DOX)-Induced Cardiac Toxicity (P00777)

Responsible Division: Systems Biology
Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination
Collaborating FDA Center: CDER
Objective(s):
1) Measure hematological parameters (white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration) to determine DOX-induced anemia.

2) Measure plasma level of cardiac troponin T as an indicator of cardiac tissue injury.
3) Identify cardiac lesions by light microscopy.

PI: Doerge, Daniel R., Ph.D.

Di(2-ethylexl)phthalate (DEHP) and Bisphenol A (BPA) Exposure in Pediatric Patients (E0742501)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CDRH
Objective(s):

1) Determine the pharmacokinetics of the production of urinary metabolites of DEHP and BPA after cardiopulmonary bypass (CPB) in children.
2) In a pilot study, quantify the exposure of children to DEHP and BPA while undergoing CPB compared to critically ill children without cardiac surgery and healthy controls.
3) Evaluate the ability of urinary biomarkers to detect acute kidney injury in patients following CPB.

PI: Ferguson, Sherry A., Ph.D.

Comorbidity of Alzheimer's Disease (AD) and Type 2 Diabetes (T2DM) in African Americans: Comparison of Biomarkers of Inflammation in Human Tissues (E0754101)

Responsible Division: Neurotoxicology
Collaborating Division: Systems Biology
Objective(s):

1) Determine the potential ethnicity-related differences in cytokine profiles of African Americans and Caucasians comorbid for AD and T2DM.
2) Examine the role of ethnicity-related insulin signaling and oxidative stress signaling in AD/T2DM tissues with specific focus on AGEs and the potential interaction with amyloid beta peptide.
3) Determine the correlations between those cytokines measured in brain tissue and those that can be measured in more accessible tissues. Endpoints measured in postmortem brain tissue provide the necessary data for fulfilling objectives 1 and 2 above; however, they cannot serve as potential biomarkers of AD. If such direct measures are strongly correlated with measures in serum or adipose tissue, then the utility of the serum markers increases substantially.

PI: Ferguson, Sherry A., Ph.D.

Preliminary Quantification of the Neurotoxicity/Neuroprotection of Selective Estrogen Receptor Modulator (SERM) Treatment in a Female Mouse Model of Alzheimer's Disease Utilizing Alzheimer Brain-Derived Amyloid ß-Protein (E0752301)

Responsible Division: Neurotoxicology
Collaborating Division/Office: Systems Biology, Office of Scientific Coordination
Objective(s):

1) Establish simultaneous use of MRS (Magnetic Resonance Spectroscopy) and microdialysis techniques.
2) Quantify the effects of raloxifene on neurochemical and behavioral endpoints.

PI: Ferguson, Sherry A., Ph.D.

Quantification of the Neuroprotection/Neurotoxicity of Long-term Selective Estrogen Receptor Modulator (SERM) Treatment in a Female Animal Model of Alzheimer's Disease (AD) (E0743401)

Responsible Division: Neurotoxicology
Collaborating Divisions: Biochemical Toxicology, Microbiology, Systems Biology

Objective(s):

Determine to what extent:

a) SERMs affect plaque deposition and behavioral deterioration in this model.
b) The effects of SERMs compare to those of E.
c) The effects of SERMs differ in the presence of endogenous E (as expressed in behavior and plaque deposition).

PI: Fisher, Jeffrey W. , Ph.D.

Biological Based Dose-Response (BBDR) Modeling for the Thyroid Axis in the Fetus and Neonate (E0743601)

Responsible Division: Biochemical Toxicology

Objective(s):

1) Create BBDR models for the hypothalamic–pituitary–thyroid (HPT) axis in the developing rat and human as a function of iodide status.
2) Interface the BBDR-HPT models with physiologically-based pharmacokinetic (PBPK) or thymidine kinase TK models for thyroid-active chemicals to predicted conditions (iodide status and chemical exposure) for which brain thyroid-hormone homeostasis cannot be maintained in the fetus and neonate.
3) Evaluate the possible influence of population exposures to thyroid-active chemicals on fetal and neonatal-thyroid status as a function of iodide intake using the models.

PI: Fisher, Jeffrey W. , Ph.D.

Biological Based Dose-Response (BBDR) Modeling for the Thyroid Axis in the Fetus and Neonate (E0743611)

Responsible Division: Biochemical Toxicology

Collaborating Divisions: Genetic and Molecular Toxicology, Systems Biology

Objective(s):

Extract raw human data on urinary excretion of iodide in infants from files provided by authors.

PI: Fuscoe, James, Ph.D.

Evaluation of Transcriptomics-Based Predictions of Sex and Age-Related Susceptibilities to Treatment-Induced Adverse Effects in F344 Rats (E0755501)

Responsible Division: Systems Biology

Collaborating Divisions: Biochemical Toxicology, Bioinformatics and Biostatistics

Collaborating FDA Center: CDER

Objective(s):

1) Identify, using bioinformatics approaches with existing data, drugs/chemicals that may exert differences in susceptible populations (e.g., young vs. adults).
2) Conduct in-life studies to confirm or refute these predictions. These studies will result in advancing a mechanistic basis for predicting *in vivo* outcomes as well as address gaps in our understanding of sex- and age-related differences in drug-induced adverse effects.

PI: Fuscoe, James, Ph.D.

Genetic and Epigenetic Mechanisms of Sex Differences in the Kidney of a Rat Model System: Developing Safety Biomarkers for FDA-Regulated Products (E0743901)

Responsible Division: Systems Biology

Collaborating Division: Bioinformatics and Biostatistics

Objective(s):

1) Perform whole-genome expression profiling on the 10 rat tissues of both sexes at 9 ages.
2) Perform miRNA profiling of selected tissues, including liver.
3) Perform DNA methylation profiling of selected tissues, including liver.
4) Use bioinformatics and statistical approaches to understand the genetic machinery operational at each developmental stage in each sex and relate the findings to potential susceptibility to adverse drug reactions and disease.
5) Use bioinformatics approaches to analyze the findings for potential age- and sex-related susceptibility in an animal model system to humans.

PI: Gamboa Da Costa, Goncalo, Ph.D.

Animal Models of Pregnancy To Address Medical Countermeasures for Influenza and Chemical, Biological, Radiological and Nuclear Threats in the "At Risk " Population of Pregnant Women—Phase I (E0746201)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Genetic and Molecular Toxicology, Bioinformatics and Biostatistics
Collaborating FDA Center: CDER
Objective(s):

1) Conduct literature search and analysis regarding animal models of pregnancy.
2) Hold public workshop.

PI: Gamboa Da Costa, Goncalo, Ph.D.

ADDENDUM to E0746201: Effect of Pregnancy on the Pharmacokinetics of Oseltamivir Phosphate (OP) and Oseltamivir Carobylate (OC) in Nonhuman Primates, Phase 2: Method Development (E0746211)

Responsible Division: Biochemical Toxicology
Collaborating Divisions/Office: Bioinformatics and Biostatistics, Genetic and Molecular Toxicology, Neurotoxicology, Office of Scientific Coordination
Collaborating FDA Center/Office: CDER, OWH
Objective(s):

1) Determine the pharmacokinetics of OP/OC in the nonpregnant nonhuman primate.
2) Determine the pharmacokinetics of OP/OC in the pregnant nonhuman primate during the first, second, and third trimester of pregnancy.
3) Determine if lactation (first two months after parturition) affects the pharmacokinetics of OP/OC in the nonhuman primate.
4) Monitor biological markers to determine the effect of pregnancy and lactation on individual biomarkers.
5) Compare the pharmacokinetics of OP/OC in nonpregnant, pregnant, and lactating nonhuman primates to the pharmacokinetics of OP/OC in nonpregnant, pregnant, and lactating humans.

PI: Gamboa Da Costa, Goncalo, Ph.D.

ADDENDUM to E0746201: Effect of Pregnancy on the Pharmacokinetics of Oseltamivir Phosphate and Oseltamivir Carobylate in Nonhuman Primates, Phase 3 (E0746231)

Responsible Division: Biochemical Toxicology
Collaborating Divisions/Office: Bioinformatics and Biostatistics, Genetic and Molecular Toxicology, Neurotoxicology, Office of Scientific Coordination

Collaborating FDA Center: CDER
Objective(s):
Determine the pharmacokinetics of oseltamivir phosphate and oseltamivir carboxylate in nonpregnant and pregnant Rhesus macaques of Indian origin.

PI: Gregori, Luisa, Ph.D.

Rapid and Sensitive Detection of Creutzfeldt-Jakob Disease Agents in Tissue and Blood Donations (E0748901)

Responsible Division: Systems Biology
Collaborating FDA Center/Office: CBER, Office of Chief Scientist Challenge Grant
Objective(s):

1) Develop a Variant Creutzfeldt-Jakob disease (vCJD) PrPTSE prototype test for blood donations.
2) Develop a prototype cornea donor test using brain-biopsy material.

PI: Hart, Mark E., Ph.D.

Co-Display of Hemagglutinin (HA) and CD154 on the Surface of Yeast Cells as a Vaccine Against Avian Influenza (E0733301)

Responsible Division: Microbiology
Objective(s):

1) Generate HA surface-presented yeast recombinant avian influenza vaccines.
2) Characterize humoral and cellular-mediated immune responses of yeast vaccines in mice.
3) Demonstrate protection of mice from lethal avian-influenza virus through yeast-based immunization.

PI: Hart, Mark E., Ph.D.

Evaluation of Methods Used to Measure Growth of *Staphylococcus aureus* and the Production of Toxic Shock Syndrome Toxin-1 (TSST-1) as Influenced by Menstrual Tampons (E0754401)

Responsible Division: Microbiology
Collaborating FDA Center: CDRH
Objective(s):

1) Utilize the tampon sac, flask-shaking, syringe, and toroid models as published to determine the ability of a variety (based upon absorbency, wadding material and design) of market-available tampons to:
   a. enhance the growth of TSST-1 producing strains of *S. aureus* isolated from clinical cases of TSS, and
   b. increase the production of TSST-1.
   Experimentally investigate parameter differences between the aforementioned methods and the results they generate to determine a standardized method for evaluating tampons for their potential to enhance *S. aureus* growth and increase TSST-1 production.

PI: Howard, Paul C., Ph.D.

ADDENDUM to E0746201: Development of a Physiologically Based Pharmacokinetic Model to Determine the Pharmacokinetic Profiles of Oseltamivir Phosphate and Oseltamivir Carboxylate in Pregnant Women (E0746221)

Responsible Office: Office of Scientific Coordination
Objective(s):

1) Provide support for analysis of the photoactivation of nanomaterials using the OH/coumarin-3-carboxylic acid assay.
2) Provide particle-size analysis for all materials being analyzed by OH method and other nanomaterials used in studies at FDA's NCTR and ORA/Arkansas Regional Laboratory.
3) Improve the assay using ultraviolet light diode laser as a replacement to the

existing broad-band ultraviolet light-A source.

PI: Imam, Syed Z., Ph.D.

Modulation of the Effects of Parkinson's Disease (PD) Medications by Nicotine (E0746601)

Responsible Division: Neurotoxicology
Objective(s):

1) Evaluate the effect of nicotine treatment on the effect of PD medications in MPP+ treated DAN human dopaminergic cells and SHSY-5Y human neuroblastoma cell lines.
2) Evaluate the effect of nicotine administration on the effects of PD medications in an MPTP-treated C57BLJ6 mouse model of progressive PD.

PI: Leakey, Julian E., Ph.D.

Subchronic Toxicity Studies of Chondroitin Sulfate and Glucosamine in Fischer 344 Rats and Diabetic Goto-Kakizaki Rats (E0215701)

Responsible Office: Office of Scientific Coordination
Objective(s):

1) Investigate the potential toxicity of chondroitin sulfate and glucosamine, administered by oral gavage in male rats.
2) Determine whether subchronic exposure of glucosamine or chondroitin sulfate potentiate the pathological effects of noninsulin-dependent diabetes in obese diabetic rats.

PI: Leakey, Julian E., Ph.D.

ADDENDUM to E0215701: Sub-Chronic Toxicity Studies of Glucosamine and Glucosamine/Chondroitin Sulfate Combinations in Obese and Lean Zucker Rats—Amended Doses (E0215711)

Responsible Office: Office of Scientific Coordination
Collaborating Division: Systems Biology
Objective(s):
Determine whether sub-chronic exposure of glucosamine or glucosamine/chondroitin sulfate combinations potentiate the pathological effects of non-insulin dependent diabetes in obese diabetic rats. The initial sub-chronic studies are necessary to establish:

a) Dose levels for the future chronic studies.
b) Whether glucosamine and/or chondroitin sulfate do stimulate TGF (Beta) and CCN2 expression in rodents.
c) Whether any observed effects of these chemicals are more severe in obese, diabetic rodents.

PI: Liachenko, Serguei, Ph.D.

Gender Differences in Neuronal Reward Circuit Activation by Nicotine and Tobacco Smoke Using Magnetic Resonance Spectroscopy (E0751001)

Responsible Division: Neurotoxicology
Collaborating Division/Office: Bioinformatics and Biostatistics, Office of Scientific Coordination
Collaborating FDA Center/Office: CTP, Office of Women's Health
Objective(s):

1) Determine biomarkers of smoking addiction and harm and describe the sex differences in these biomarkers. These biomarkers can then be used to provide more effective and personalized smoking cessation treatments based on gender and level of addiction. For example, nicotine replacement therapies seem less effective in women.
2) Describe neurometabolite changes in

response to acute and sub-acute exposure to nicotine or tobacco smoke.

3) Noninvasively measure neurometabolite changes—increasing the translational nature of the outcomes.

PI: Lumen, Annie, Ph.D.

Population-Based Computational Framework for Assessing Xenobiotic Disposition and Interaction Effects in Pregnant Women—Pilot Study (E0752201)

Responsible Division: Biochemical Toxicology

Collaborating Division: Bioinformatics and Biostatistics

Collaborating FDA Office: Office of Women's Health

Objective(s):

1) Develop a population-based integrated Physiologically Based Pharmacokinetic (PBPK) Biological Based Dose-Response (BBDR) model for the hypothalamic–pituitary–thyroid axis during pregnancy to predict the pharmacokinetics of iodide, perchlorate, and their interactions on serum thyroid hormone levels of pregnant women.
2) Create an extension of the PBPK-BBDR pregnancy model to evaluate serum-thyroid hormone perturbations from concomitant exposures to multiple thyroid active agents found in food and the environment, such as thiocyanate, in addition to perchlorate and iodide, in a pregnant population.

PI: Lyn-Cook, Beverly A., Ph.D.

Clinical and Biological Significance of Three Identified Targets in Systemic Lupus Erythematosus Patient PBMCs: IL-18, TNFSF13B, and FoxP3 (E0744611)

Responsible Division: Biochemical Toxicology

Objective(s):

1) Determine expression levels of toll-like receptors 3, 7, 9, and miRNA-146a in controls and lupus patients grouped according to sex and ethnicity.
2) Correlate expression of TRLs 3,7,9 to type-1 interferon levels in lupus and control patients.
3) Determine the polymorphisms profile of 3,7,9 in lupus and control patients and correlate to expression levels.
4) Correlate expression levels of TRLs 3,7,and 9 with expression of miRNA-146a.
5) Determine if interferon regulation may be through epigenetic regulation.

PI: Lyn-Cook, Beverly A., Ph.D.

Male vs. Female Expression in Human Liver, Kidney, and Small Intestine: Microarray Analyses (E0743001)

Responsible Division: Biochemical Toxicology

Collaborating Division: Systems Biology

Objective(s):

1) Ascertain whether there are basic expression differences in drug metabolizing tissues (liver, kidney, and small bowel) between males and females. These studies will be conducted on untreated tissue (no drugs). The major focus will be on drug-metabolizing enzymes and transporters particularly. An assessment of major biological pathways that display sexual dimorphism will also be made.
2) Answer the question: "Do functional differences between male and female tissues exist in the liver?" Explore this with the liver sandwich assay and several drugs that display sexual dimorphism in the clinic. These drugs

include paclitaxel, rosiglitazone, and pioglitazone initially (the list may expand).

PI: Lyn-Cook, Beverly A., Ph.D.

Sex and Ethnic Differences in Expression of Toll-Like Receptors (TLR-3, TLR-7, and TRL-9) in Systemic Lupus Erythematous: New Targets for Emerging Therapeutics (E0744601)

Responsible Division: Biochemical Toxicology

Objective(s):

1) Determine the expression levels of TLRs 3,7, 9, and miRNA-146a in controls and lupus patients grouped according to sex and ethnicity.
2) Correlate expression of TLRs 3,7, 9 to type 1 interferon levels in lupus and control patients.
3) Determine the polymorphisms profile of TLRs 3,7, and 9 in lupus and control patients and correlate to expression levels.
4) Correlate expression levels of TRLs 3,7, and 9 with expression of miRNA-146a.
5) Determine if interferon regulation may be through epigenetic regulation.

PI: Lyn-Cook, Beverly A., Ph.D.

The Role of Sex in Expression of DNA Cytosine 5-Metyltransferases, Histone Deacetylases, Acetylases, Metyltransferases, and Demethylases Among Patients with Systemic Lupus Erythematosus (SLE): Elucidating Potential New Drug Targets (E0738601)

Responsible Division: Biochemical Toxicology

Objective(s):

Elucidate whether there is a sex and/or ethnic bias in expression levels of epigenetic markers in SLE patients.

PI: Myers, Meagan B., Ph.D.

Determining Oncomutation Profile of Triple Negative Breast Cancer: Information to Direct Development of Personalized Therapies (E0743801)

Responsible Division: Genetic and Molecular Toxicology

Collaborating FDA Center/Office: CDRH, Office of Women's Health

External Partner: University of Arkansas for Medical Sciences

Objective(s):

1) Establish which molecules should be targeted to treat the largest percentages of breast cancers.
2) Identify which mutational biomarkers should be used as diagnostics in personalized approaches to breast-cancer treatment.
3) Determine what sensitivity is needed in the measurement of those mutational biomarkers.

PI: Ning, Baitang, Ph.D.

ADDENDUM to E0745301: Identification of Genetic Variants Responsible for Carbamazepine (CBZ) - Induced Adverse Drug Reactions in Han Chinese (E0745311)

Responsible Division: Systems Biology

Collaborating Division: Bioinformatics and Biostatistics

Collaborating FDA Center: CDER

Objective(s):

Identify genetic variants responsible for individual differences in drug responses.

PI: Ning, Baitang, Ph.D.

Genetic Variants in Cardiovascular Disease Risks and Drug Responses: Exome Sequencing and Variant Characterization in the Amish Population (E0752601)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Bioinformatics and Biostatistics, Systems Biology
Objective(s):

1) Identify causative alleles for four phenotypic traits that were associated with risks of cardiovascular disease, based on previously obtained genetic markers and association studies.
2) Perform validation genotyping assays in existing cohorts consisting of approximately 1000 well-phenotyped individuals to further confirm risk-association variants.
3) Conduct functional assessments using biochemical approaches to delineate mechanisms underlying the association between the variants and phenotypic traits.

PI: Ning, Baitang, Ph.D.

Whole-Genome Sequencing to Identify Genetic Susceptibilities to Carbamazepine-Induced Adverse Reactions (E0745301)

Responsible Division: Systems Biology
Collaborating Divisions: Bioinformatics and Biostatistics, Neurotoxicology
Objective(s):

1) Identify and compare genetic variants in 30 patients with Steven-Johnson Syndrome/toxic epidermal necrolysis and 10 Amish individuals with public data from the 1000 Genome Project.
2) Identify genetic variants associated with phenotypes of interest.
3) Evaluate the molecular mechanisms accounting for interindividual variations responding to carbamazepine.
4) Model patient-specific drug/human leukocyte antigen interactions to predict the outcome.
5) Assess technical performance and bioinformatics solutions of next-generation sequencing on whole-genome sequencing.

PI: Pang, Li, Ph.D.

Sex Differences in Drug-Induced QT Prolongation and Torsade de Pointes (TdP): Establishing an *In vitro* Model for High-Throughput Screening and Risk Assessment of Torsadogenic Drugs (E0754001)

Responsible Division: Biochemical Toxicology
Collaborating Division: Systems Biology
Collaborating FDA Center: CDER
External Partner: University of Arkansas for Medical Sciences
Objectives:

1) Establish the model and positive control.
2) Evaluate the sensitivity and specificity of the model and test the possibility of high-throughput screening and ranking QT prolonging drugs for the risk of TdP.

PI: Pang, Li, Ph.D.

The Role of ABC-Drug Transporters in Chemoresistance in Pancreatic Cancer (E0751101)

Responsible Division: Biochemical Toxicology
Objective(s):

1) Compare various ABC transporters' expression in normal and pancreatic adenocarcinoma specimens and determine whether the expression of ABC transporters is correlated with

clinical aggressiveness of the tumor.
2) Evaluate whether the single nucleotide polymorphisms in ABC transporters genes are associated with the abnormal expression of the efflux pumps and drug sensitivity.
3) Assess the epigenetic regulation of ABC transporters in pancreatic cancer.

PI: Parsons, Barbara L., Ph.D.

Cancer Mutations as Biomarkers of Cancer Risk: Human Studies with Implications for Personalized Medicine (E0726501)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Bioinformatics and Biostatistics
Objective(s):

1) Develop the information necessary for the rational use of oncogene mutations as quantitative biomarkers of cancer risk; specifically Allele-specific competitive blocker PCR (ACP-PCR) will be used to determine normal and pathological levels of relevant oncogene mutations in multiple human tissues and tumors.
2) Compare the information derived from human tissues with data generated in a parallel rodent protocol as an approach for incorporating carcinogenesis-relevant data into the rodent to human extrapolation necessary in cancer-risk assessment.
3) Validate a streamlined ACP-PCR methodology and develop the methodology necessary to measure oncogene mutant fraction in cell-free DNA isolated from plasma.
4) Convey to the regulatory risk-assessment community through a series of publications, the regulatory significance of the data regarding tumor-associated mutations which have and will be generated.

PI: Parsons, Barbara L., Ph.D.

Improving the Efficacy and Development of Targeted Cancer Therapeutics by Establishing a Model to Identify Molecularly-Targeted Therapies that Prevent Acquired Resistance (E0755101)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Systems Biology
Objective(s):

1) Establish a 3D cancer tissue-originated spheroid model at NCTR, which can be used to experimentally assess the development of acquired drug resistance (expected to occur with monotherapy).
2) Assess the development of drug-resistant clones by total spheroid area, cell cycle profile, the frequency of apoptosis, and by ACB-PCR (a method that can quantify minor mutant subpopulations).
3) Determine, using the model, which combination of molecularly-targeted chemicals inhibits growth of NSCLC spheroids to the greatest extent. Treatments will include erlotinib alone and in combination with drugs/chemicals expected to target KRAS mutant cells. This project will employ a genomics approach (RNA sequencing) to identify additional genetic lesions that impact response to treatment.

PI: Paule, Merle G., Ph.D.

Complex Brain Function in Children as Measured by Performance in the NCTR Operant Test Battery (E0703301)

Responsible Division: Neurotoxicology

Objective(s):
Measure aspects of learning, short-term memory and attention, motivation, time perception, color, and position discrimination.

PI: Progribny, Igor P., Ph.D.

Development of a Targeted MicroRNA-Based Epigenetic Approach for Breast Cancer Treatment (E0746101)

Responsible Division: Biochemical Toxicology
Collaborating Division: Bioinformatics and Biostatistics
Objective(s):

1) Show that assessing expression of microRNAs (miRNAs) that target DNA methyltransferases and other components of DNA methylation machinery will improve diagnosis of breast cancer.
2) Demonstrate that correcting miRNA function will restore the expression of epigenetically silenced genes in breast cancer, inhibit breast-cancer progression, and improve the therapeutic efficacy of existing conventional chemotherapeutic drugs.

PI: Sarkar, Sumit, Ph.D.

Evaluation and Characterization of Blood-Brain Barrier (BBB) Pathology in MPTP-Probenecid-Induced Parkinsons Disease (PD)-Like Conditions in a Mice Model and its Potential Amelioration by Endoplasmic Reticulum Stress Reducers (Molecular Chaperones) and Other Putative Anti-PD Therapeutics (E0751201)

Responsible Division: Neurotoxicology
Collaborating Division/Office: Systems Biology, Office of Scientific Coordination
Objective(s):

1) Determine the role of key neurovascular units in the expression of PD-like pathology.
2) Determine the ability of endoplasmic reticulum stress-reducers to alter the expression of PD-like pathology.
3) Determine the ability of antioxidant peptide SS31, N-acetyl cysteine, acetyl-l-carnitine, the orexin A receptor inhibitor SB 334 867 A, and metal chelators, such as M30, clioquinol, and VK-28 to provide neuroprotection against PD-like pathology.
4) Evaluate changes in cerebral hemodynamics, BBB permeability and neurochemicals associated with the development of PD-like pathology.
5) Conduct behavioral assessments for those compounds shown to be efficacious in ameliorating PD pathology to quantify symptom improvement.

PI: Shi, Qiang, Ph.D.

Identifying Drugs That Cause Female-Biased Hepatotoxicity by Analyzing FDA Drug-Approval Packages/Labels and FDA-Maintained Databases and Conducting Comparative Studies in Primary Hepatocytes of Rats, Mice, and Humans (E0750201)

Responsible Division: Systems Biology
Collaborating FDA Office: Office of Women's Health
Objective(s):

1) Identify specific drugs that cause drug-induced liver injury (DILI) more often in women than in men.
2) Establish a hepatocyte culture model to study a drug's potential to induce sex-biased DILI.

PI: Varma, Vijayalakshmi, Ph.D.

Epigenetics, DNA Methylation, and Obesity (E0733101)

Responsible Division: Systems Biology

Collaborating Division/Office:
Biochemical Toxicology, Office of Scientific Coordination
Objective(s):
Evaluate the effect of differences in DNA methylation and agouti signaling protein in the offspring of Avy/a dams x a/a sires as a result of nutrient x gene interactions. These preliminary data will be used to select the appropriate diets for further studies on obesity and type 2 diabetes.

PI: Varma, Vijayalakshmi, Ph.D.

Viable Yellow Agouti Mouse Breeding Colony (S00763)

Responsible Division: Systems Biology
Objective(s):
Maintain the Viable Yellow Agouti Mouse breeding colony at NCTR, to provide animals needed to support studies addressing research questions on:

a. Understanding the role of epigenetic and genetic mechanisms in health and disease states.
b. Understanding the developmental origin of adult diseases in response to toxicants, drugs, or nutrients.
c. Understanding the differences in disease susceptibilities of lean and obese phenotypes/non-diabetic and diabetic phenotypes or non-neoplastic and neoplastic phenotypes having similar genotype.
d. Investigating potential differences in drug toxicities between lean and obese/non-diabetic and diabetic/non-neoplastic and neoplastic states.

PI: Wagner, Robert, Ph.D.

Immunological Effects of Nanoparticles on Induction of Pro-inflammatory Responses to *Candida albicans* by Vaginal Epithelial Cells (VEC) (E0752001)

Responsible Division: Microbiology
Collaborating FDA Office: ORA
Objective(s):

1) Measure the effects of graphene and PLGA nanoparticles on mRNA and protein expression of inflammatory cytokines and signal transduction proteins by VEC stimulated with *C. albicans*.
2) Measure oxidative effects and DNA damage in VEC by nanoparticles.
3) Evaluate effects of nanoparticles on estrogen receptor-mediated signal transduction and suppressed cytokine responses using estrogen receptor inhibitor ICI182780 and an estrogen receptor reporter assay.

PI: Williams, Denita, Ph.D.

ADDENDUM to E0746201: Development of a Physiologically Based Pharmacokinetic Model to Determine the Pharmacokinetic Profiles of Oseltamivir Phosphate and Oseltamivir Carboxylate in Pregnant Women (E0746221)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Bioinformatics and Biostatistics, Genetic and Molecular Toxicology
Collaborating FDA Center: CDER
Objective(s):
Develop a physiologically based pharmacokinetic model that reflects human physiological values for use in determining the pharmacokinetic profiles of oseltamivir phosphate and

oseltamivir carboxylate during pregnancy.

PI: Yang, Xi, Ph.D.

MicroRNA (miRNA) as Noninvasive Biomarkers for Tobacco Smoke-Associated Bladder Cancer (E0751801)

Responsible Division: Systems Biology
Collaborating Division: Bioinformatics and Biostatistics
Collaborating FDA Product Center: CTP
Objective(s):

1) Determine if miRNA in biofluids and/or tissues can be used to assess the bladder-cancer risk posed by tobacco products. Biofluid (blood and urine) miRNA levels from patients (smokers and non-smokers) with bladder cancer and normal healthy controls will be compared to identify specific miRNAs that may serve as new biomarkers of tobacco smoking-induced bladder cancer.
2) Analyze the tumor and matched normal tissue samples from the same patient to correlate such miRNA biomarkers between tissue and biofluids.

PI: Zhang, Yongbin, Ph.D.

Development of Methods for Determining Nanoparticle Penetration/Permeation into Vaginal Mucosal Tissue (E0750701)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Offices: ORA, Office of Women's Health
Objective(s):

1) Obtain many samples of feminine hygiene products that claim to have nanomaterials, and develop the methodologies to detect and characterize the nanomaterials.
2) Develop/adapt methods to determine nanoscale material penetration in rat vaginal mucosal tissue to determine if there is a potential hazard that requires further investigation.

FY 2014 Publications

Publication is an essential component of research. All documents authored by NCTR investigators must undergo the NCTR Document Review and Approval Process, which consists of the review, clearance, and approval by the Center Director prior to submitting the publication to a journal. The list below identifies the NCTR-approved publications that were **accepted or published in journals in FY 2014, and book chapters that were accepted in FY 2014**.

- Ahn, Y., Kim, J. and Cerniglia, C.E. (2014). Evaluation of liquid and solid culture media for the recovery and enrichment of *Burkholderia cenocepacia* from distilled water. *Journal of Industrial Microbiology and Biotechnology*, 41:1109-1118.

  Responsible Division: Microbiology

- Ahn, Y., Stuckey, R.V., Sung, K., Rafii, F. and Cerniglia, C.E. (2013). Influence of sterilized human fecal extract on the sensitivity of *Salmonella* enterica ATCC 13076 and *Listeria* monocytogenes ATCC 15313 to enrofloxacin. *Antibiotics*, 2:485-499.

  Responsible Division: Microbiology

- Bae, D., Mezal, E., Smiley, R., Cheng, C. and Khan, A.A. (2014). The sub-species characterization and antimicrobial resistance of *Listeria* monocytogenes isolated from domestic and imported food products from 2004 to 2011. *International Journal of Food Microbiology*, 64:656-663.

  Responsible Division: Microbiology

- Bearden, D.W., Beger, R., Broadhurst, D., Dunn, W., Edison, A., Guillou, C., Trengove, R., Viant, M. and Wilson, I. (2014). The New Data Quality Task Group (DQTG): ensuring hgh quality data today and in the future. *Metabolomics*, 10:539-540.

  Responsible Division: Systems Biology

- Bhattacharyya, S., Yan, K., Pence, L.M., Simpson, P.M., Gill, P., Letzig, L., Beger, R., Sullivan, J.E., Kearns, G.L., Reed, M.D., Marshall, J.D., Van Den Anker, J.N. and James, L.P. (2014). Targeted liquid chromotography-mass spectrometry analysis of serum acylcarnities in acetaminophen toxicity in children. *Biomarkers in Medicine*, 8(2):1-12.

Responsible Division: Systems Biology

- Bisgin, H., Liu, Z., Fang, H., Kelly, R., Xu, X. and Tong, W. (2014). A phenome-guided drug repositioning through a latent variable model. *BMC Bioinformatics*, 15:267.

  Responsible Division: Bioinformatics and Biostatistics

- Boros, L.G., Beger, R., Linehan, W., Farkas, Jr., G., Somlyai, G., Girnun, G., Biswal, S. and Meuillet, E.J. (2014). Targeted 13C-labeled tracer fate associations for drug efficacy testing in cancer. *Tumor Cell Metabolism - Pathways, Regulation and Biology*, 15: 363-371

  Responsible Division: Systems Biology

- Camacho, L.M., Basavarajappa, M.S., Chang, C., Han, T., Kobets, T., Koturbash, I., Surratt, G. Lewis, S.M., Vanlandingham, M., Fuscoe, J., Gamboa Da Costa, G., Pogribny, I.P. and Delclos, K.B. (2014). Effects of bisphenol A on the status of global genomic DNA methylation and gene expression in the prostate, female mammary gland, and uterus of NCTR Sprague-Dawley rats treated orally from gestation day 6 until postnatal day 4 or 90. *Toxicological Sciences*, 139(1):174-197.

  Responsible Division: Biochemical Toxicology

- Cao, X., Mittelstaedt, R.A., Pearce, M.G., Allen, B.C., Hernandez, L.G., Johnson, G.E., Bigger, C.H. and Heflich, R.H. (2014). Quantitative dose-response analysis of ethyl methanesulfonate genotoxicity in adult gpt-delta transgenic mice. *Environmental and Molecular Mutagenesis*, 55:385-399.

  Responsible Division: Genetic and Molecular Toxicology

- Chelonis, J., Prunty, P.K., Cox, A.R., Paule, M.G. and Karr, M.J. (2014). Comparison of delayed matching-to-sample performance in monkeys and children. *Behavioural Processes*, 103:261-268.

  Responsible Division: Neurotoxicology

- Chen, C., Chen, J.J., (2013). Benchmark dose calculation for ordered categorical responses. *Risk Analysis*, 34(8):1435-1447.

  Responsible Division: Bioinformatics and Biostatistics

- Chen, J.J., Lu, T., Chen, D. and Wang, S.J. (2014). Biomarker adaptive designs in clinical trials. *Translational Cancer Research*, 3:279-292.

  Responsible Division: Bioinformatics and Biostatistics

- Chen, M., Bisgin, H., Tong, L.J., Hong, H., Fang, H., Borlak, J.T. and Tong, W. (2014). Toward predictive models for drug-induced liver injury in humans: Are we there yet? *Biomarker in Medicine*, 8 (2):201-213.

  Responsible Division: Bioinformatics and Biostatistics

- Chen, M., Borlak, J.T. and Tong, W. (2014). Predicting idiosyncratic drug-induced liver injury: Some recent advances. *Expert Review of Gastroenterology & Hepatology*, 8(7):721-723.

  Responsible Division: Bioinformatics and Biostatistics

- Chen, M., Kelly, R., Tong, L.J., Borlak, J.T. and Tong, W. (2013). Phenotypic anchoring of liver injury in rats by gene expression profiling. *Microarrays: Principles, Applications and Technologies*, Nova Science Publishers, 11: 211-230.

  Responsible Division: Bioinformatics and Biostatistics

- Chen, M., Tung, C., Fang, H., Shi, Q., Guo, L., Shi, L., Borlak, J.T. and Tong, W. (2014). A testing strategy to predict risk for drug-induced liver injury in humans using high-content screen assays and the "rule-of-two" model. *Archives of Toxicology*, 88:1439-1449.

  Responsible Division: Bioinformatics and Biostatistics

- Chen, S., Melchior, W.B., Wu, Y. and Guo, L. (2014). Autophagy in drug-induced liver toxicity. *Journal of Food and Drug Analysis*, 32(1):83-104.

  Responsible Division: Biochemical Toxicology

- Chen, S., Xuan, J., Couch, L.H. and Guo, L. (2014). Sertraline induces endoplasmic reticulum stress in hepatic cells. *Toxicology*, 322:78-88.

  Responsible Division: Biochemical Toxicology

- Chen, S., Xuan, J., Wan, L., Lin, H., Couch, L.H., Mei, N., Dobrovolsky, V.N. and Guo, L. (2013). Sertraline, an antidepressant agent, induces apoptosis in hepatic cells through mitogen-activated protein kinase pathway. *Toxicology Sciences*, 13-0661.

Responsible Division: Biochemical Toxicology

- Chen, T., Moore, M.M. (2014). The mouse lymphoma assay. *Genotoxicity and DNA Repair: a practical approach*, Humana Press, 19: 323-342.

  Responsible Division: Genetic and Molecular Toxicology

- Chen, T., Yan, J. and Li, Y. (2014). Genotoxicity of titanium dioxide nanoparticles. *Journal of Food and Drug Analysis*, 22:95-104.

  Responsible Division: Genetic and Molecular Toxicology

- Chien, C., Tsai, C., Chang, C. and Chen, J.J. (2014). MAVTgsa: an R package for multivariate analysis of variance test for gene set analysis. *BioMed Research International*, 2014:1-11, http://dx.doi.org/10.1155/2014/346074.

  Responsible Division: Bioinformatics and Biostatistics

- Churchwell, M.I., Camacho, M., Vanlandingham, M., Twaddle, N.C., Sepehr, E., Delclos, K.B., Fisher, J.W. and Doerge, D.R. (2014). Is life stage-dependent internal dosimetry for Bisphenol A consistent with an estrogenic mode of action in Sprague Dawley rats when compared with reference estrogen, ethinyl estradiol, and endogenous estradiol? *Toxicological Sciences*, 139(1): 4-20. doi: 10.1093/toxsci/kfu021.

  Responsible Division: Biochemical Toxicology

- Consortium, S. (2014). A comprehensive assessment of RNA-seq accuracy, reproducibility and information content by the Sequence Quality Control consortium. *Nature Biotechnology*, 32(9):903-917.

  Responsible Division: Bioinformatics and Biostatistics

- Cui, L., He, Z., Ferguson, S.A. and Paule, M.G. (2014). On the role of PDE4D in cerebral ischemia. *Journal of Neurological Disorders and Stroke*, 2(3):1066-10752.

  Responsible Division: Neurotoxicology

- Delclos, K.B., Camacho, M., Lewis, S.M., Vanlandingham, M., Latendresse, J.R., Olson, G.R., Davis, K.J., Patton, R.E., Gamboa Da Costa, G., Woodling, K.A., Bryant, M.S., Chidambaram, M., Trbojevich, R., Juliar, B., Felton, R.P. and Thorn, B.T. (2014). Toxicity evaluation of Bisphenol A administered by gavage to

Sprague Dawley rats from gestation day 6 through postnatal day 90. *Toxicological Science*, 139:174-197.

Responsible Division: Biochemical Toxicology

- Delclos, K.B., Churchwell, M.I., Camacho, M., anlandingham, M., Twaddle, N.C., Sepehr, E., Delclos, K.B., Fisher, J.W. and Doerge, D.R. (2014). Comparison of life-stage-dependent internal dosimetry for Bisphenol A, ethinyl estradiol, a reference estrogen, and endogenous estradiol to test an estrogenic mode of action in Sprague Dawley rats. *Toxicological Sciences*, 139:4-20.

  Responsible Division: Biochemical Toxicology

- Ding, W., Bishop, M.E., Pearce, M.G., Davis, K.J., White, G.A., Lyn-Cook, L.E. and Manjanatha, M. (2014). Sex-specific dose-response analysis of genotoxicity in cyproterone acetate-treated F344 rats. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis*, 744:1-7.

  Responsible Division: Genetic and Molecular Toxicology

- Dobrovolsky, V.N., Heflich, R.H. (2014). The human RBC PIG-A gene mutation assay. *Genotoxicity and DNA Repair*, Humana Press, 10: 169-184

  Responsible Division: Genetic and Molecular Toxicology

- Erickson, B.D., Elkins, C., Mullis, L., Heinze, T.M., Wagner, R.D. and Cerniglia, C.E. (2014). A metallo-B-lacatamase is responsible for the degradation of Ceftiofur by the bovine intestinal bacterium *Bacillus cereus* P41. *Veterinary Microbiology*, 172:499-5040.

  Responsible Division: Microbiology

- Fang, H., Su, Z., Wang, Y., Miller, A., Liu, Z., Howard, P., Tong, W. and Lin, S.M. (2014). Extracting and combining unique health information from electronic databases: exploring the FDA Adverse Event Reporting System (FAERS) for disease monitoring. *Clinical Pharmacology and Therapeutics*, 95(5):496-498.

  Responsible Division: Bioinformatics and Biostatistics

- Ferguson, S.A., Law, C.D. (2014). Effects of perinatal methylphenidate (MPH) treatment on postweaning behaviors of male and female Sprague Dawley rats. *Neurotoxicology and Teratology*, 42:9-16.

  Responsible Division: Neurotoxicology

- Ferguson, S.A., Law, C.D. and Kissling, G.E. (2014). Developmental treatment with ethinyl estradiol, but not Bisphenol A, causes alterations in sexually dimorphic behaviors in male and female Sprague Dawley rats. *Toxicological Sciences*, 140:374-392.

  Responsible Division: Neurotoxicology

- Fu, P.P. (2014). Introduction: nanomaterials - toxicology and medical applications. *Journal of Drug and Food Analysis*, 11(1):1-2.

  Responsible Division: Biochemical Toxicology

- Gollapudi, B., Lynch, A.M., Heflich, R.H., Dertinger, S.D., Dobrovolsky, V.N., Froetschi, R., Horibata, K., Kenyon, M.O., Kimoto, T., Lovell, D., Stankowski Jr., L.F., White, P., Witt, K.L. and Tanir, J. (2014). The *in vivo* Pig-A assay: A Report of the International Workshop on Genotoxicity Testing (IWGT) Workgroup. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis*, 2014:1-13.

  Responsible Division: Genetic and Molecular Toxicology

- Gong, B., Wang, C., Su, Z., Hong, H., Auerbach, S., Shi, L., Tong, W. and Xu, J.Z. (2014). Transcriptomic profiling of rat liver samples in a comprehensive study design by RNA-Seq. *Scientific Data*, 1:140021. doi:10.1038/sdata.2014.21.

  Responsible Division: Bioinformatics and Biostatistics

- Guo, X., Zhang, S., Dial, S.L., Boudreau, M.D., Xia, Q., Fu, P.P., Levy, D.D., Moore, M. and Mei, N. (2014). *In vitro* investigation of the mutagenic potential of Aloe Vera extracts. *Toxicology Research*, 3 (6):487-496.

  Responsible Division: Genetic and Molecular Toxicology

- He, G., Laundry, M., Chen, H., Thorpe, C., Walsh, D., Varela, M.F. and Pan, H. (2014). Detection of benzalkonium chloride resistance in community environmental isolates of *Staphylococci*. *Journal of Medical Microbiology*, 63:735-741.

  Responsible Division: Microbiology

- He, W., Wamer, W., Xia, Q., Yin, J. and Fu, P.P. (2014). Enzyme-like activity of nanomaterials. *Journal of Environmental Science and Health, Part C: Environment Carcinogen, Ecotoxicology Review*, 32(2):186-211.

Responsible Division: Biochemical Toxicology

- Holland, R.D., Wilkes, J.G., Cooper, W.M., Alusta, P.S., Williams, A.J., Pearce, B.A., Beaudoin, M.A. and Buzatu, D.A. (2014). Thymol treatment of bacteria prior to matrix-assisted laser desorption/ionization time-of-flight mass spectrometric analysis aids in identifying certain bacteria at the subspecies level. *Rapid Communication in Mass Spectrometry*, 28:2617-2626.

  Responsible Division: Systems Biology

- Hong, H., and Tong, W. (2014). Emerging efforts for discovering new biomarkers of liver disease and hepatotoxicity. *Biomarkers in Medicine*, 8(2):143-146.

  Responsible Division: Bioinformatics and Biostatistics

- Ibberson, C.B., Jones, C.L., Singh, S., Wise, M.C., Hart, M.E., Zurawski, D.V. and Horswill, A.R. (2014). *Staphylococcus aureus* hyaluronidase is a CodY-regulated virulence factor. *Infection and Immunity*, 82(10):4253-4264.

  Responsible Division: Microbiology

- Inselman, A.L., Hansen, D.K. (2014). Developmental toxicity of antiepileptic drugs – An update. Reference Module in *Biomedical Sciences*. Comprehensive Toxicology, Volume 12, 2010 (177-187).

  Responsible Division: Systems Biology

- Inselman, A.L., Nolen, G., Chang, C., Harrouk, W., Fisher, E., Tassinari, M.S. and Hansen, D.K. (2013). Re-evaluation of the embryonic stem cell tTest. *International Journal of Regulatory Sciences*, 1(1): 32-49.

  Responsible Division: Systems Biology

- Johnson, G.E., Hernandez, L.G., Gollapudi, B., Pottenger, L.H., Bodger, O., Dearfield, K.L., Lovell, D., Heflich, R.H., Hixon, G.J., MacGregor, J.T., Thompson, C.M., Abraham, L., Thybaud, V., Zeiger, E., Benthem, J. and Whitten, P. (2014). Derivation of points of departure (PoD) estimates in genetic toxicology studies and their potential application in risk assessment. *Environmental and Molecular Mutagenesis*, 55(8): 609-623.

  Responsible Division: Genetic and Molecular Toxicology

- Kanungo, J., Cuevas- Martinez, E.Y., Ali, S.F. and Paule, M.G. (2014). Zebrafish model in drug safety assessment. Advances in Developmental and Reproductive

Toxicology - Special Issue of the *Journal of Current Pharmaceutical Design*, 20:1-14.

Responsible Division: Neurotoxicology

- Khare, S., Williams, K.M., Bekele, A. and Gokulan, K. (2014). Human intestinal microbial biofilm and its correlation with intestinal mucin secretion. *Biofilms in the Food Environment*. ISBN: 9781118864142

  Responsible Division: Microbiology

- Kheradmand, E., Rafii, F., Yazdi, M., Sepahi, A.A., Shahverdi, A.R. and Oveisi, M.R. (2014). The antimicrobial effect of selenium nanoparticle-enriched probiotics and their fermented broth against *Candida albicans. DAUR Journal of Pharmaceutical Sciences*, 22(1): 48.

  Responsible Division: Microbiology

- Koturbash, I., Melnyk, S.B., Beland, F.A. and Pogribny, I.P. (2013). Role of epigenetic and miR-22 and miR-29b alterations in the downregulation of Mat1a and Mthfr genes in early preneoplastic livers in rats induced by 2-acetylaminofluorene. *Molecular Carcinogenesis*, 52:318-327.

  Responsible Division: Biochemical Toxicology

- Kweon, O., Kim, S., Kim, D., Kim, J., Kim, H., Ahn, Y., Sutherland, J.B. and Cerniglia, C.E. (2014). Pleiotropic and epistatic behavior of a ring-hydroxylating oxygenase system in the polycyclic aromatic hydrocarbon metabolic network from *Mycobacterium vanbaalenii* PYR-1. *Journal of Bacteriology*, 196 (19):3503-3515.

  Responsible Division: Microbiology

- Lain, C.G., Xu, S., Guo, W., Yan, J., Frank, M., Liu, C., Chen, Y., Murphy, G.F. and Chen, T. (2014). Decrease of 5-hydroxymethylcytosine in rat liver with subchronic exposure to genotoxic carcinogens riddelliine and aristolochic acid. *Molecular Carcinogenesis*, doi: 10.1002/mc.22201.

  Responsible Division: Genetic and Molecular Toxicology

- Lantz-McPeak, S.L., Guo, X., Cuevas- Martinez, E.Y., Dumas, M.A., Newport, G.D., Ali, S.F., Paule, M.G. and Kanungo, J. (2014). Developmental toxicity assay using

high content screening of zebrafish embryos. *Journal of Applied Toxicology,* 35(3): 261-272.

Responsible Division: Neurotoxicology

- Lee, M., Liu, Z., Kelly, R. and Tong, W. (2014). Of text and gene: Using text mining methods to uncover hidden knowledge in toxicogenomics. *BMC Systems Biology*, 8:93: http://www.biomedcentral.com/1752-0509/8/93.

  Responsible Division: Bioinformatics and Biostatistics

- Lin, G., Li, N., Ruan, J., Fu, P.P. and Zhao, Z. (2014). Chemical diversity investigation of hepatotoxic pyrrolizidine alkaloids in qianliguang (senecio scandens) and related species by uplc-qtof-ms. *Journal of Pharmaceutical and Biomedical Analysis*.

  Responsible Division: Biochemical Toxicology

- Lin, H., Guo, X., Zhang, S., Dial, S.L., Guo, L., Manjanatha, M., Moore, M. and Mei, N. (2014). Mechanism evaluation of ginkgo biloba leaf extract-induced genotoxicity in L5178Y cells. *Toxicological Sciences*, 10.1093: toxsci/kfu037.

  Responsible Division: Genetic and Molecular Toxicology

- Liu, Y., Ai, N., Keys, A.A., Fan, X. and Chen, M. Network pharmacology for traditional Chinese medicine research: Methodologies and applications. *Chinese Herbal Medicine*.

  Responsible Division: Bioinformatics and Biostatistics

- Lu, T., Chuang, E.Y. and Chen, J.J. (2013). Identification of reproducible gene expression signatures in lung adenocarcinoma. *BMC Bioinformatics*, 10.1186. Accepted: 41628. Project #: E0740301, Tracking #: 33315

  Responsible Division: Bioinformatics and Biostatistics

- Luan, Y., Liu, M., Qi, X., Xie, X., Wu, M., Xu, L., Chen, T. and Ren, J. (2014). Absence of mature miRNAs inactivates the response of gene expression to carcinogenesis induced by N-ethyl-N-nitrosourea in mouse liver. *Journal of Applied Toxicology*, 34(12):1409-1417.

  Responsible Division: Genetic and Molecular Toxicology

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Glossary of Acronyms and Abbreviations

This glossary is provided to assist you in interpreting acronyms, abbreviations, and phrases you encounter while reading this publication. This is not meant to take the place of standard language or scientific dictionaries, which should be referred to if any short form of a scientific term does not appear in this glossary. Also, you may refer to the Index of Key Terms, located at the end of this publication, as a quick reference to locate other occurrences of a specific term.

Acronym/ Abbreviation	Name
ACH	Arkansas Children's Hospital
AD	Alzheimer's Disease
ADRA	Associate Director for Regulatory Activities
Ag	Silver
APAP	Acetaminophen
ARL	Arkansas Regional Laboratory
BBB	Blood-brain barrier
BBDR	Biologically based dose-response
BPA	Bisphenol A
CBER	Center for Biologics Evaluation and Research, FDA
CDER	Center for Drug Evaluation and Research, FDA
CDRH	Center for Devices and Radiological Health, FDA
CFSAN	Center for Food Safety and Applied Nutrition, FDA
CoV	Coronaviruses
CRADA	Cooperative Research and Development Agreement
CT	Computed tomography
CTP	Center for Tobacco Products, FDA
CVM	Center for Veterinary Medicine, FDA
DA	Dopamine
DEHP	Di-(2-ethylhexyl)phthalate
DILI	Drug-induced liver injury
ED	Endocrine disruptor
EDKB	Estrogen Disruptor Knowledge Base
ENM	Engineered nanomaterials
ENU	N-ethyl-N-nitrosourea
ES	Embryonic stem cells

Acronym/ Abbreviation	Name
GABA	Gamma-aminobutyric acid
GWAS	Genome-Wide Association Study
HESI	Health and Environmental Sciences Institute
HHS	Department of Health and Human Services
HPHC	Harmful and potentially harmful constituents
IAG	Interagency agreement
IDR	Idiosyncratic drug reactions
IHC	Immunohistochemistry
ILSI	International Life Sciences Institute
in silico	Modeled on a computer
in situ	In place; localized and confined to one area
In vitro	In animal models
In vivo	In cell cultures
IPS	Induced Pluripotent Stem cell line
LILI	Leflunomide-Induced Liver Injury
LO	Liver Ontology
LTKB	Liver Toxicity Knowledge Base
MALDI	Matrix-assisted laser desorption ionization
MAQC	MicroArray Quality Control
mEST	Mouse Embryonic Stem Cell Test
miRNA	MicroRNA
MOA	Mode-of-action
MOU	Memorandum of Understanding
MPH	Methylphenidate hydrochloride
MPP+	(1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NASH	Nonalcoholic steatohepatitis
NCTR	National Center for Toxicological Research, FDA
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NMDA	N-methyl-d-aspartate
NMR	Nuclear magnetic resonance

Acronym/ Abbreviation	Name
NP	Nanoparticle
NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
OMH	Office of Minority Health, FDA
ORA	Office of Regulatory Affairs, FDA
OSC	Office of Scientific Coordination, FDA/NCTR
OWH	Office of Women's Health, FDA
PBPK	Physiologically based pharmacokinetic
PCR	Polymerase chain reaction
PD	Parkinson's Disease
PET	Positive emission tomography
PI	Principal Investigator
PIG-A	Phosphatidylinositol glycan anchor biosynthesis, class A
PND	Post-natal day
QSDAR	Quantitative Spectrometric Data-Activity Relationship
RAPID-B™	Rapid Identification of Bacterial Pathogens
RBC	Red blood cell
RET	Reticulocytes
ROS	Reactive-oxygen species
SAB	Science Advisory Board
SHLC	Sex hormone-like compound
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
SSL	Simulated-solar light
TCKB	Tobacco Constituents Knowledge Base
TERA	Toxicology Excellence for Risk Assessment
TiO2	Titanium dioxide
TSST-1	Toxic-Shock Syndrome Toxin-1
UALR	University of Arkansas at Little Rock
VCJD	Variant Creutzfeldt-Jakob Disease
VGDS	Voluntary Genomic Data Submission
VXDS	Voluntary eXploratory Data Submission
ZnO	Zinc oxide

Index of Principal Investigators/Division Directors

Ahn, Young-Beom, Ph.D. 108,144,151
Ali, Syed F., Ph.D. 81,136,150,151
Azevedo, Marli P., Ph.D. 108,157
Bearden, Ted 109
Beger, Richard D., Ph.D. 82,129,144,145,154, 157,158,159
Beland, Frederick A., Ph.D. 29, 82, 151
Binienda, Zbigniew K., Ph.D. 82
Boudreau, Mary Ph.D 83,84,149,159
Bowyer, John, Ph.D. 109,157
Buzatu, Dan A., Ph.D. 109,150,157
Camacho, Luisa M., Ph.D. 145,147,148,156
Cao, Xuefei, Ph.D. 84,145
Cerniglia, Carl E., Ph.D. 55,85,144,148,151,153,159
Chang, Ching-Wei, Ph.D. 129,130,145,147,150,159
Chelonis, John J., Ph.D. 110,130,131,145
Chen, Huizhong, Ph.D. 85,149,155
Chen, James J., Ph.D. 85,110
Chen, Minjun, Ph.D. 131,146,152,160
Chen, Tao, Ph.D. 110,111,151,152,154,159,160
Delclos, Kenneth B., Ph.D. 85,86,145,147,148,156
Desai, Varsha G., Ph.D. 112,131
Ding, Wei, Ph.D. 113,148
Dobrovolsky, Vasily N., Ph.D. 113,146,148,149,156
Doerge, Daniel R., Ph.D 86,87,113,132,147,148,155,161
Fang, Jia-long, Ph.D. 88
Ferguson, Sherry A, Ph.D. 88,132,147-149,155,156
Fisher, Jeffrey W., Ph.D. 89,133,147,148,150
Foley, Steven L., Ph.D. 89,157
Fu, Peter P., Ph.D. 113,114,149,152,156,161
Fuscoe, James, Ph.D. 133,145,153,155,156,159-161
Gamboa Da Costa, Goncalo, Ph.D. 134,145
George, Nysia, Ph.D. 89
Goodwin, Amy K., Ph.D. 89
Gough, Bobby J., Ph.D. 90
Gregori, Luisa, Ph.D. 135
Gu, Qiang, Ph.D. 90,114,115
Guo, Lei, Ph.D. 152,160
Guo, Xiaoqing, Ph.D. 91,149,151,152
Hammons, George J., Ph.D. 91,155
Hanig, Joseph P., Ph.D. 115,157
Hansen, Deborah K., Ph.D. 92,93,116,150
Harris, Steven, Ph.D. 93
Hart, Mark E., Ph.D. 135,150,157
He, Zhen, Ph.D. 116,147
Heflich, Robert H., Ph.D. 46,93,94,116,145,148-150
Hiranata, Takato, Ph.D. 94
Hong, Huixiao, Ph.D. 94,116,146,149,150,155,159-161
Howard, Paul C., Ph.D. 6,9,12,95,117-119,135,146,148,149,150,155,159-161
Hu, Shu-Chieh, Ph.D. 17,95,96
Imam, Syed Z., Ph.D. 136
Inselman, Amy L., Ph.D. 119,150
Kanungo, Jyotshnabala, Ph.D. 96,97,119,120,150,151
Khan, Ashraf A., Ph.D. 119,120,144,154,159
Khan, Saeed A., Ph.D. 97,120,153
Khare, Sangeeta, Ph.D. 97,120,151,154,159
Kim, Seong-Jae, Ph.D. 98,121,151
Leakey, Julian E., Ph.D. 98,99,121,136
Liachenko, Serguei, Ph.D. 121,136
Liu, Zhichao, Ph.D. 121,145,148,152,160
Lumen, Annie, Ph.D. 137
Lyn-Cook, Beverly A., Ph.D. 137,138,148,155
Manjanatha, Mugimane, Ph.D. 99,121,122,148,152-154
Mattes, William B., Ph.D. 74,99,122,153,159
McDaniel, Lea Patrice 99
McKinzie, Page, Ph.D. 122
Meehan, Joseph F., Ph.D. 100,159,161
Mendrick, Donna L., Ph.D. 3,28,153,158-160
Mei, Nan, Ph.D. 100,101,146,149,152,155,160
Mittelstaedt, Denita, Ph.D. 100,145,156
Myers, Meagan B., Ph.D. 138,154
Nakamura, Noriko, Ph.D. 100,122
Nayak, Rajesh R., Ph.D. 101
Ning, Baitang, Ph.D. 138,139,154,155,160
Pang, Li, Ph.D. 139,155
Paredes, Angel, Ph.D. 123
Parsons, Barbara, Ph.D. 102,140,154
Perkins, Roger G. 123,155,160,161
Paule, Merle G., Ph.D. 40,68,102,103,140,145,147,150,151

Petibone, Dayton, Ph.D. 124,156
Progribny, Igor P., Ph.D. 141
Rafii, Fatemeh, Ph.D. 104,144,151
Sarkar, Sumit, Ph.D. 141,157
Schmued, Laurence C., Ph.D. 157
Schnackenberg, Laura, Ph.D. 124,158,160
Shi, Qiang, Ph.D. 124,141,146,159,160
Slavov, Svetoslav, Ph.D. 125,157,158
Su, Zhenqiang, Ph.D. 148,149,155,159-161
Sung, Kidon, Ph.D. 104,144,153
Sutherland, John B., Ph.D. 125,151,156
Tolleson, William H., Ph.D. 104,125,155
Tong, Weida, Ph.D. 36,104,125,126,145,146,148-150,152,155,158,159-161
Trbojevich, Raul, Ph.D. 105,147,158
Varma, Vijayalakshmi, Ph.D. ... 126,141,142,159
Wagner, Robrt, Ph.D. 142,148
Wang, Cheng, Ph.D. 105,106,149,159-161
Wang, Yuping, Ph.D. 127,148,158,159
Wilkes, Jon G., Ph.D. 127,150,157
Williams, Denita, Ph.D. 142
Yang, Xi, Ph.D. 106,143,153,158-160
Yu, Li-Rong, Ph.D. 127
Zhang, Xuan, Ph.D. 107,157
Zhang, Yongbin, Ph.D. 143,155

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